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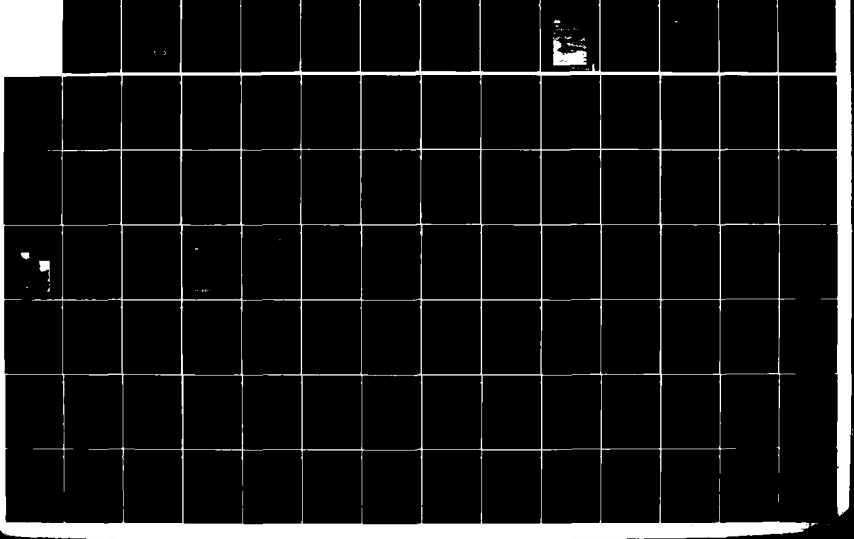

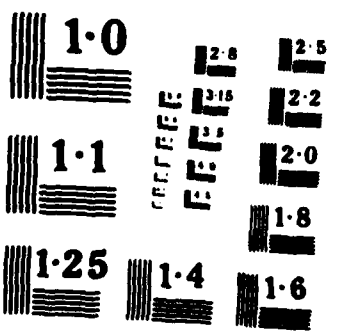
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SYMPOSIA SUMMARY

10th International RES Congress

Ito, Japan

September 2-7, 1984

GRANT # N00014-84-G-0189

Submitted To: Office of Naval Research

From: Sherwood M. Reichard
President, IURES
Medical College of Georgia
Augusta, GA 30912

The opening address of the 10th International RES Congress, Ito, Japan, was given by Dr. Zanvil Cohn of the Rockefeller Institute, NYC, NY, USA. Dr. Cohn reviewed the many facets of macrophage function which secure for this cell a central role in both the stimulation and expression of immune responses. Among the topics reviwed by Dr. Cohn was the variety and complexity of effector activities expressed by macrophages, both in a resting state and following stimulation with soluble products released at sites of inflammation and immune reactions. Many of these effector reactions require interaction of the stimulating agent with receptors present on the macrophage cell membrane. Some receptors, such as the antibody (Fc) receptors, induce not only internalization of the immune complex, but secretion of a variety of reactive substances (complement components, clotting factors, enzymes, reactive O_2^-, etc.); other receptors, such as the complement (C3) and mannosyl receptors, stimulate only phagocytosis. Regulation of phagocytosis/secretion may be through alteration in free (non-mitochondrial) calcium. One secretory product that induces death of certain intracellular microorganisms and extracellular tumor targets is reactive O_2^-. A soluble product from stimulated T lymphocytes that induces the secretion of reactive oxygen intermediates is IFN_{gamma}. This molecule appears to regulate a whole host of macrophage effector activities, and deficiencies in IFN_{gamma} production have been correlated with development of certain progressive diseases (i.e. leprosy). Although macrophages clearly participate in the regulation and expression of secondary

immune responses, Dr. Cohn felt that the evidence from Dr. Steinman's laboratory strongly suggest that dendritic cells, cells derived from a different progenitor than macrophages, have primary responsibility for antigen presentation to T cells in developing immune responses. This viewpoint was challenged several times throughout the meeting.

PLENARY SESSIONS

Viruses, Oncogenes, and Human Lymphomas

The definition of an oncogene depends upon one's perspective: one can view these genes as either a cellular gene incorporated into a virus, or a virus gene incorporated into a human chromosome. The development of neoplastic disease following exposure to certain viruses is a complex process. For example, the infection of African children with EBV virus does not necessarily result in development of lymphomas: these infected children are at risk for development of lymphomas, but the virus infection is not sufficient in itself. Rather, chronic endemic malaria is the environmental co-factor that induces splenomegally and sets the stage for induction of Burketts lymphoma. EBV then activates and immortalizes the B cells in a BCGF (B cell growth factor)-independent manner (Klein, USA).

Every cellular body carries greater that 20 protooncogenes. The method of transformation activation depends upon the particular gene: transduction into acute transforming retroviruses, integration, insertional mutagenesis (acquisition of more promoters), chromosomal rearrangement, gene amplification, single point mutations, and other methods as yet undescribed. A wide variety of human tumors (bladder, colon, gall bladder, liver, lung, pancreas, fibrosarcoma, rhabdosarcoma) have detectible oncogenes, mostly of the K *ras* type. The *ras* genes are so named because of the similarity with rat sarcoma virus, and there are 3 types: H, N, and K. The K *ras* gene codes for a single 21 kbase peptide, and malignancy results from a single point mutation in the 12th or the 61st codon. Is activation of oncogenes essential for malignancy, or simply coincidental? In all cases examined, both in humans and experimental models, it is the single mutation of guanosine to adenine at position 12 that is associated with mammary tumor development. This substitution may be the result of a methylation reaction (Sukumar, USA).

There is a high level of adult T cell leukemia in south central Japan. Cells from these patients can be cultured without addition of TCGF (T cell growth factor), and produce virus as well as shed ATLV antigens. Serum from patients react with these viral antigens, and the antigens can be detected on the surface of T cells. Over 70% of the lymphomas diagnosed in southern Japan are ATVL-induced T cell lymphomas. Most of the patients are over 40 years old, and there appears to be family clustering. The virus may be passed from mother to child (Hanoaka, Japan).

Macrophages and Atherosclerosis

IL-2, a T cell-derived T cell growth promoting factor, induces the release of clotting factors that enhance the adhesion of monocytes to endothelium. Monocytes constitute a fair number of the cells in plaque that leads to atherosclerosis (Catran, USA).

Some foam cells (lipid-containing cells that are associated with plaque formation in arteries) derive from macrophages, others from smooth muscle tissue. The correlation between premature atherosclerosis and lipids appears to be with elevated low-density lipoprotein (LDL), yet macrophages accumulate LDL too slowly to account for development of foam cells. Acetylation, or chemical modification, converts LDL into a molecule that can be rapidly accumulated by macrophages. There is no evidence that de novo chemical modification occurs in vivo, but these workers show evidence that endothelial cell-modified LDL is rapidly internalized by a receptor that does not recognize the "native" molecule. Smooth muscle tissue can also modify the LDL, but most other cells cannot. This modification is accompanied by the peroxidation of fatty acids, and is dependent upon the generation of oxygen radicals. EDTA blocks the modification. Apparently the Apoprotein B of LDL is the site of modification, as well as fatty acid phosphorylation. Monocytes constituitively release lipoprotein lipase, and activation of this enzyme by apoprotein CII induces the accumulation of cholesterol esters and triglycerides. The question is: Is the macrophage a good guy (ingestion of LP and prevention of lesion) or a bad guy (ingestion of LDL, formation of foamy cells, and development of lesion)? (Steinberg, USA)

Receptor-mediated internalization of LDL occurs primarily (70%) through coated pits, although coated pits are only 2% of the cell surface. There are

several naturally-occurring defects in receptor-mediated entry of LDL that have facilitated analysis of the process of internalization and accumulation of LDL: (1) defective clustering in coated pits (loss of C-terminal end of receptor) and (2) defective binding. Clathrin is the major protein in coated pits. Depletion of K^+ destroys coated-pit distribution in cells, and induces a decrease in LDL uptake. Receptor-LDL uncoupling must occur for recycling of the LDL receptor. The current theory is that uncoupling occurs in endosomes prior to lysosome fusion; uncoupling appears to be pH directed, since monensin and cloroquin raise the internal pH of endosomes and decrease reinsertion of LDL receptors (Anderson, USA).

Macrophages and Activation

Tissue macrophages encounter a wide variety of signals during inflammation and development of immune responses. The burning questions about macrophage activation today are: what is the nature of the signal(s) that induce activation? what are the capacities that the macrophage must acquire for the various effector activities displayed? and, what is the molecular basis of macrophage activation? In all tumor killing assays, binding of the target cell to the macrophage is an essential event. Binding via a receptor triggers the release of a proteolytic enzyme of 38-40 kdaltons that kills the target. The binding of the tumor cell and release of the proteolytic enzyme during nonantibody-mediated macrophage killing are events that are independently genetically regulated. In the presence of antibody, some activated macrophages can kill certain tumor targets by release of reactive oxygen intermediates (classic ADCC). Some macrophages that are <u>not</u> activated can also mediate ADCC, albeit a slower form of this killing. Thioglycollate macrophages are the best example of cells that can perform this slow ADCC. A molecular correlate of macrophage response to activating signals is phosphorylation by protein kinase c (PKc). One can mimic the priming of macrophages by IFN_{gamma} for extracellular destruction of tumor targets with phorbol myristate acetate (PMA) and a Ca^{++} ionophore. Both priming sequences require an additional trigger signal (such as LPS) to induce cytotoxicity. In both instances, protein kinase c is increased during priming, and LPS provides the trigger for protein phosphorylation (Adams, USA).

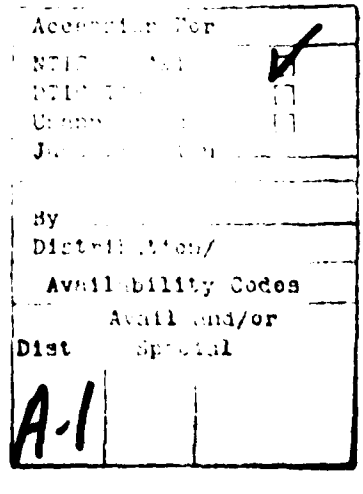
Although monoclonal antibodies have been made that interact with a variety of macrophage subtypes, none of these antibodies are specific for the activated macrophage. F4/80 is a monoclonal that recognizes a macrophage surface antigen that is down-regulated during activation (was produced by S. Gordon). These investigators reported a new monoclonal, ACM-1, that identifies activated macrophages only: it is not reactive with inflammatory macrophages, or resident macrophages of the peritoneum, spleen, or lungs, with spleen cells or with thymocytes. The antibody recognizes an antigen with 2 polypeptides of 70 and 45 kdaltons. It blocks cytotoxicity, but does not eliminate cytotoxic cells in the presence of complement. It is expressed on BCG, C. parvum and pyran-activated macrophages (Taniyama, Japan).

Interferon gamma is a major activating factor for macrophages of both humans and mice. As little as 1 pM of IFN_{gamma} is sufficient to activate these cells. There is a great deal of information that suggests that the tumoricidal and microbicidal properties of activated macrophages can be correlated with the release of certain reactive oxygen intermediates, notably hydrogen peroxide. This molecule is responsible, in whole or in part, for the destruction of tumor cells and obligate intracellular parasites. These investigators have been able to show a deficiency in IFN_{gamma} production in humans and experimental animals during debilitating chronic diseases such as leprosy and leishmaniasis, and have begun preliminary trials with replacement therapy using recombinant IFN_{gamma} in mice infected with these agents. One exciting finding is a marker for interferon therapy: neopterin (derives from GTP in folate or serotonin system) is excreted in urine of patients treated with this macrophage activating agent (Nathan, USA).

The antiviral activity and macrophage activating activities of IFN_{gamma} appear to reside in different regions of the molecule. Monoclonal antibodies can be made to recombinant IFN that inhibit either one or the other of these activities in fluid phase, but both activities when attached to a solid matrix or precipitated by Staph A. Hybrid molecules of mouse and human (not active on mouse cells) IFN_{gamma} suggest that there are at least 2 domains, each of which regulate a different activity (Schreiber, USA).

That IFN_{gamma} is a major activating factor for macrophages is now clear: this molecule can induce extracellular cytolysis of diverse tumor and helminth targets, and intracellular destruction of a variety of obligate

intracellular pathogens. The question is whether there are non-IFN macrophage activating factors as well. In certain cases, non-interferon MAFs can be detected: (1) a 25 kd molecule in culture fluids of the PMA-stimulated EL-4 thymoma cell line induces potent extracellular killing of tumor and helminth targets without IFN-associated antiviral and Ia-inducing activity. This MAF activity cannot be neutralized by anti-IFN monoclonal antibodies. (2) factors are present in lymphokine supernatants of antigen or mitogen-stimulated spleen cells that induce intracellular destruction of microorganisms, but do not have antiviral activity. (3) Resistance to infection, one early antimicrobial activity of activated macrophages, cannot be induced with recombinant IFN_{gamma}, nor can this MAF activity in lymphokine supernatants be neutralized by monoclonal antibodies prepared against IFN_{gamma}. (Meltzer, USA).



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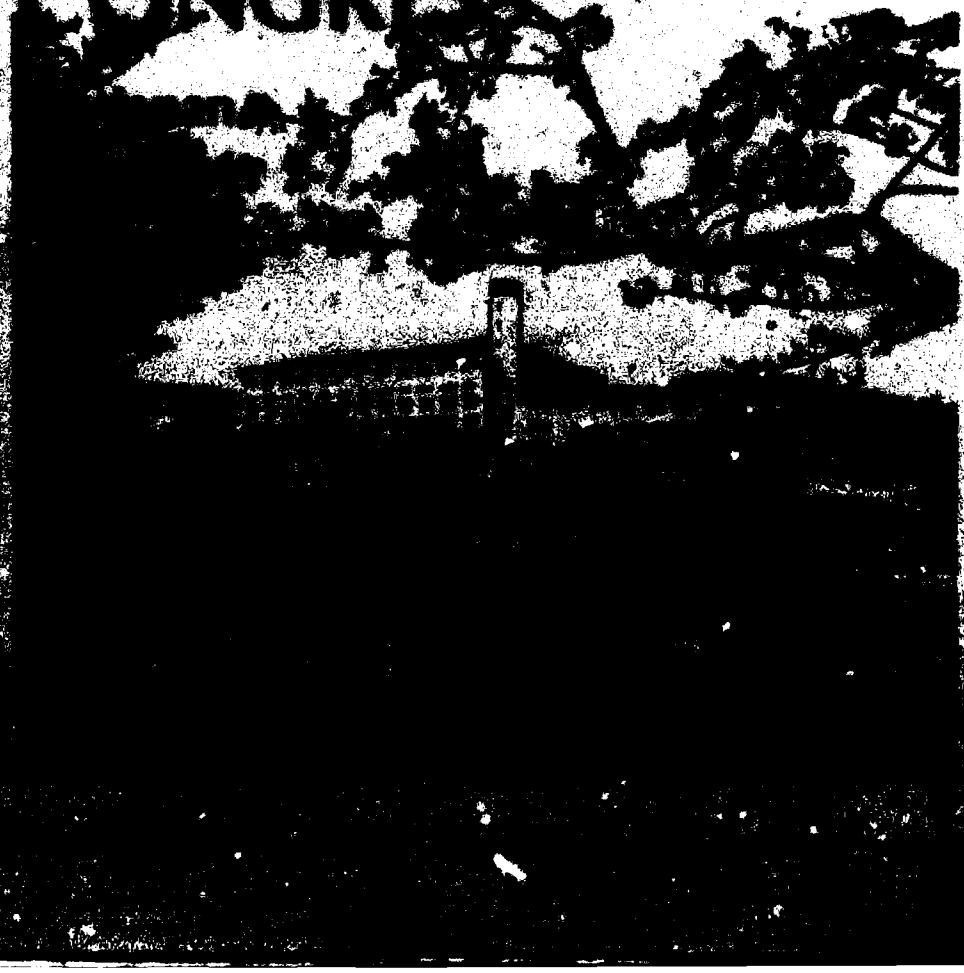
10th INTERNATIONAL RES CONGRESS



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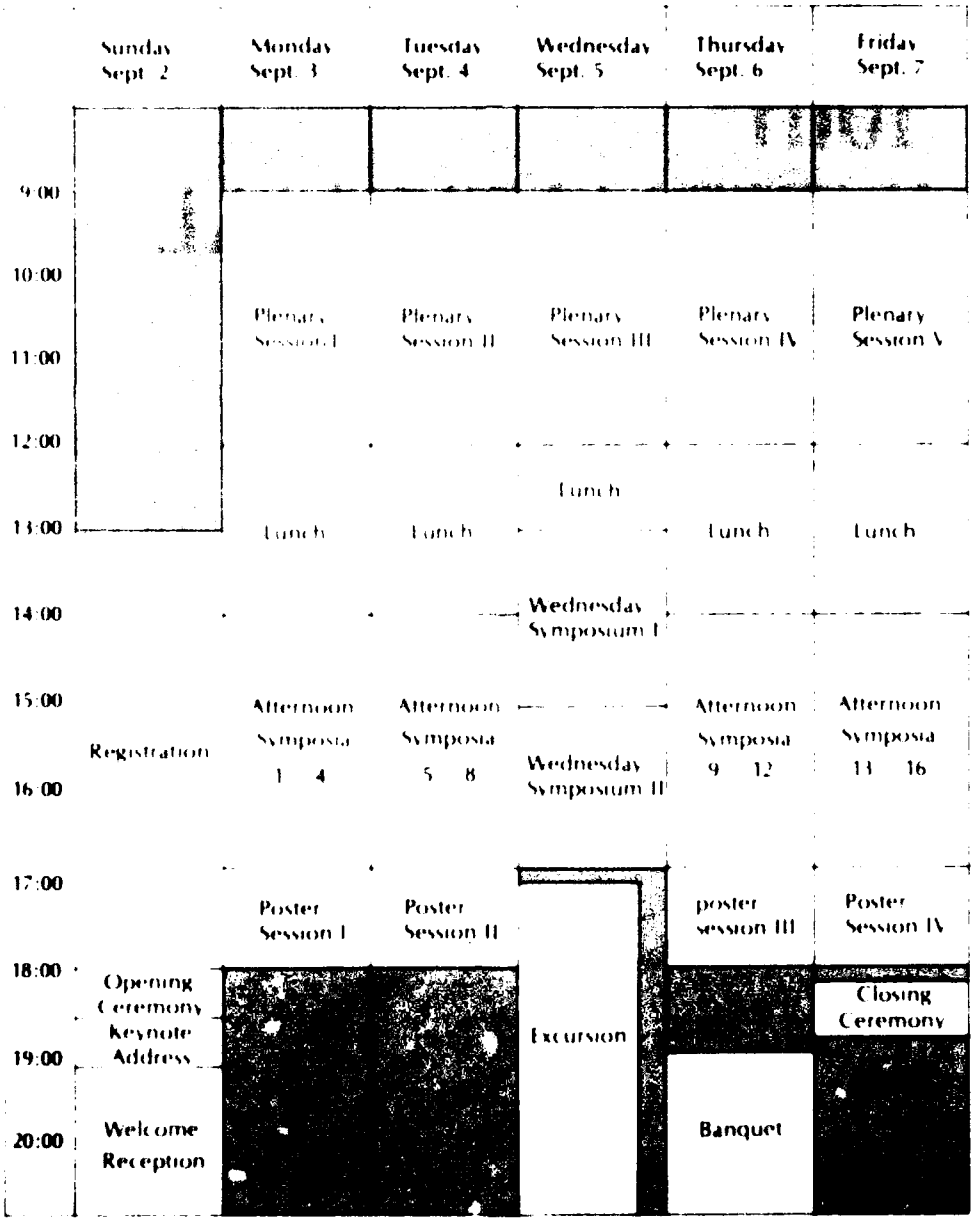
10th INTERNATIONAL RES CONGRESS

Ito, Japan
September 2-7, 1984,

PROGRAM

PROGRAM AT A GLANCE



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GENERAL INFORMATION

1. **Period and Site**

   Period: September 2 (Sunday) – September 7 (Friday), 1984

   Site: The Kawana Hotel

   Address: 1459, Kawana, Ito, Shizuoka Prefecture 414, Japan

   Telephone: 0557-45-1111

   The Kawana Hotel, one of Japan's nicest resort hotels, is on the side of the Pacific Ocean, surrounded by the beauty of Fuji-Hakone-Izu National Park.

2. **Language**

   The official language is English. There will be no facilities for simultaneous translation.

3. **Poster Session**

   Posters are on display in Room E every day except Wednesday from 13:00 to 18:00. One hour period between 17:00 and 18:00 is reserved as the "Poster Session" for free discussions with the designers.

4. **Secretariat**

   The Secretariat is open from 8:00 to 19:00 at Secretariat Room on the 1st floor throughout the Congress period. If you have any trouble, please contact the Secretariat.

5. **Information Desk**

   The General Information Desk, to be set up at the Congress, will offer answers to general questions, while the Travel Information Desk, set up by the Japan Travel Bureau, will provide information on accommodations and travel. The desks will open:

September 2, Sunday	13:00 – 19:00
September 3, Monday – September 6, Thursday	8:30 – 17:00
September 7, Friday	8:30 – 12:00

6. **Notice Board**

   A notice board will be installed at the lobby on the 1st floor for both general and special notices, and personal messages to the Congress participants.
   In case you want to get ahold of someone at the Congress, please go to the General Information Desk. They will post your notice on the Notice Board.

7. **Name Cards**

   Please display your Name Card (provided to you at the time of registration) at all times during the Congress for your convenience and security. Cards have been prepared in 4 colors as follows:

Blue	Active Member and Young Scientist
Red	Family Member
Purple	Invited Guest
Green	Secretariat

8. **Currency Exchange**
Foreign currency exchange services will be provided at the Kawana Hotel

9. **First-Aid and Medical Assistance**
Participants needing first-aid should apply to the Congress Secretariat, or the front desk of the hotel. Medical care or hospitalization can be quickly arranged in case of emergency.

10. **Sports: Golf, Tennis and Swimming**
Among the Izu Peninsula, Ito City is most frequented by foreign visitors, and The Kawana Hotel has one of the most famous Golf courses in Japan, "the Fuji course" and "the Oshima course." And the Resort Hotel Southern Cross also has very good Golf courses. You can also enjoy tennis at the above mentioned hotels. The pool may also be freely used, but be aware that no lifeguard is on duty.
The Sports Desk will be set up in the 1st floor lobby. Those wishing to participate in golf or tennis are requested to apply at this desk.

11. **Lunch**
Lunch will be served at the following locations within the Kawana Hotel:

Grill Room (Main Building, Basement)	11 00	– 15 00
Dining Room (Main Building, 1st Floor)	12 00	– 14 00
Lounge (Main Building, 1st Floor)	12 00	– 14 00
Sun Parlor (Main Building, 1st Floor)	10 00	18 00

12. **Coffee Break**
Coffee breaks are planned for both the morning and afternoon. The morning break will be in Banquet Lobby at 10 30 – 11 00. The afternoon break will be held in Room E (Poster Session Room) at 16 00 – 17 00.

13. **Excursion**
"Noh tour" is planned as an excursion on September 5, Wednesday. Asaba's Noh is very unique in that you may enjoy the traditional atmosphere associated with the most aristocratic of Japan's theater arts. For questions about or application for the excursions, please inquire at the Travel Information Desk.

SOCIAL EVENTS

1. **Opening Ceremony**
   Date: September 2, Sunday
   Time: 18:00 — 18:40
   Place: Room A
   *Following the Opening Ceremony, the Keynote Address will be presented
   Time: 18:40 — 19:30

2. **Welcome Reception**
   Date: September 2, Sunday
   Time: 19:40 — 21:00
   Place: Room C
   Fee: Included in registration fee
   Dress: Informal

3. **Banquet**
   Date: September 6, Thursday
   Time: 19:00 — 21:00
   Place: Room A, B
   Fee: Included in registration fee
   Dress: Informal

4. **Closing Ceremony**
   Date: September 7, Friday
   Time: 18:15 — 18:45
   Place: Room A

Business Meeting (IURES officers only)
Date: September 4, Tuesday
Time: 12:00 — 13:00
Place: Room H

SCIENTIFIC PROGRAM

Keynote Address — **Sept. 2 (Sun)**
Room A (18:40 – 19:40)

The Macrophage as Multifaceted Cell
Zanvil A. Cohn

Chairperson: Mizu Kohma

Plenary Session I — **Sept. 3 (Mon)**
Room A+B (9:00 – 12:00)

Viruses, Oncogenes and Human Lymphomas

Presiding: Masao Hanaoka

1. Oncogenes in Human Cancer — Saraswati Sukumar
2. The Relationship of HTLV to Lymphoma and AIDS — Salahuddin
3. The Role of Oncogenes in Lymphoid Neoplasms — George Klein
4. Viruses and Human Lymphomas — Masao Hanaoka

Symposium 1 — **Sept. 3 (Mon)**
Room A (14:00 – 17:00)

The Regulation of Macrophage Development and Function

Chairpersons: Isaiah J. Fidler and S. Kasakura

1. The Effects of the Various Agents on the Cultured Kupffer Cells — Shotaro Sakisaka
2. Phenotypic Characterization of Gamma Interferon-Induced Human Monocyte Polykaryons (MP) — J.B. Weinberg
3. Regulation of expression of LPS Receptor on Mouse Lung Macrophages by Lymphokines — K.S. Akagawa

4 Protein Kinase Activity on the Cell Surface of a Macrophage-Like Cell Line, J774.1 Cells — F. Amano

5 Selective Tumor Cell Lysis by Non Specifically Activated Macrophages Derived from Long-Term Bone Marrow Cultures — J. Loewenstein

6 Fc Receptor Modulation and Cytotoxic Activity of Porcine Pulmonary Alveolar Macrophages — Yoon B. Kim

7 The Failure of Mycobacteria to Stimulate Phagocyte Superoxide Anion Generation is Correlated with the Absence of Complement Activation in Vitro — T.J. Holzer

8 The Regulatory Role of Lipoxygenase Products on the Stimulated State of Rat Kupffer Cells — M. Birmelin

Symposium 2: **September 3 (Mon)**
Room B (14:00 – 17:00)

Analysis of Macrophage Regulation and Effector Functions

Chairpersons: J. Stephen Haskill and S. Muramatsu

1 Disappearance and Reappearance of Resident Macrophages: Importance in C. parvum Induced Tumoricidal Activity — Stephen Haskill

2 Endotoxin Induced Monocyte Macrophage Procoagulant Activity in the Rat Requires Collaborating T-Lymphocytes — Peter A. Lando

3 The Role of Spleenic Macrophage on the Blood Cell Destruction — S. Matsuda

4 The Induction of Human Monocyte Interleukin-1 Synthesis and Secretion — R.C. Newton

5 GM-CSA Production by Human Monocyte Subsets — J.R. Zucali

6 Peanut Agglutinin Receptors on the Human Macrophage-Histiocyte Series — R. Tsunoda

7 Immunohistochemical Localization of S-100 Protein Subunits in the Human Lymphoreticular System — T. Akagi

Symposium 3 **September 3 (Mon)**
Room C 14 00 17 00

The Surface and Receptors of Mononuclear Phagocytes

Chairpersons: Thomas A. Hamilton and T. Masuda

1 Effective Internalization of Polysaccharide-Coated Liposomes into Phagocytes
Yasuko Ueda

2 Evaluation of the Expression of Ia-Antigen on Normal an Immune Peritoneal Macrophages as Demonstrated by Rosetting, Immunocytochemistry and Antigen Presentation
Robert H J Beelen

3 A Comparative Study on the Presence of Antigenic Determinants on Normal Reactive and Malignant Macrophages
P J M Roholl

4 The Uptake of Polysaccharide-Coated Liposomes by Alveolar Macrophage
Akimitsu Tomonaga

5 Complementary Roles of Kupffer Cells (KC) and Liver Endothelial Cells (LEC) in the Endocytic Function of RES in the Liver
Bard Smedsrod

6 Identification, Quantitation, and Partial Characterization of a Serum Factor which Inhibits Fibronectin Collagen Binding Activity
Frank B. Gelder

7 Macrophage Surface Changes Caused by Influenza Virus and Interferon
M. Nowakowski

Symposium 4 **September 3 (Mon)**
Room D 14 00 17 00

The Kill of Microbes by Elements of the MPS

Chairpersons: Seymour J. Klebanoff and E. Ouchi

1 Adoptive Transfer of Immune Responsiveness from Heavily Infected Anergic
Frank M. Collins

2 Macrophage Activation and Resistance to Listeria monocytogenes
Maurice J. Lefford

3 Bactericidal Activity of Peritoneal Macrophages of Beige Mice with Chediak Higashi Syndrome — Masayasu Nakano

4 Electron Microscopic Study on the Interaction of Listeria monocytogenes and Subpopulations of Mouse Peritoneal Macrophages — Masaharu Kizaki

5 The Effect of Bilirubin and Bile Acids on Oxygen-Dependent Bactericidal Activity of Human Neutrophils — Masahiko Iwanaga

6 Effects of Prostaglandins and Scavengers for Oxygen Intermediates on Cytotoxicity of Polymorphonuclear Leukocytes (PMN) — Reiji Kasukawa

7 N Formyl Methionyl Leucyl Phenylalanine Induced Superoxide Release of Calcium-Depleted Human Neutrophils — Miwako Nakagawara

8 Enhancement of Oxygen Consumption of Neutrophils by Vanadate — Yukio Ozaki

Poster Session I — **Sept. 3 (Mon)**
Room E 17:00 – 18:00

Chairpersons: You Hui Zhang, M. Yamasaki and Sherwood M. Reichard

1 Protective Role of Alveolar Macrophage Enzymes in Experimental Pulmonary Tuberculosis — Saroj Chandrasekhar

2 Immunological Consequences of Host-Parasite Membrane Interactions in Human Falciparum Malaria — C. F. Ockenhouse

3 Endocytosis of the Latex Particles by the Endothelial and Kupffer Cells in the Perfused Rat Liver — Chieko Dan

4 Foamy Macrophages Associated with Erythrophagocytosis — Tokuhiro Ishihara

5 Lectin Like Receptor on Murine Macrophage Cell Line Cells, Mml. Involvement of Sialic Acid-Binding Sites in Opsonin Independent Phagocytosis for Xenogenic Red Cells — Seishi Kyoizumi

6 Uptake of Mast Cell Granules by Reticular Cells and Macrophages and Their Acid Phosphatase Activity in the Rat Lymph Node — Kenji Miyata

7 Phagosome-Lysosome Fusion in Human Macrophages. First Encounter with M leprae — David M. Scollard

Plenary Session II — **September 4 (Tue)**
Room A+B (9:00 – 12:00)

Macrophages and Atherogenesis

Presiding: Ramzi S. Cotran and Mizu Kojima

1. Macrophages, Foam Cells and Atheromas — Ramzi S. Cotran
2. Uptake of Lipids and Regulation of Cholesterol Metabolism — Richard G. Anderson
3. Macrophages and Lipoproteins — Daniel Steinberg
4. Macrophages and Secretion of Apolipoproteins — Zena Werb
5. Macrophages and Accumulation of Cholesterol Ester in Atheromatous Aorta — Tatsuya Takano

Symposium 5 — **September 4 (Tue)**
Room A (14:00 – 17:00)

The Regulation and Execution of the Inflammatory Response

Chairpersons: Sigurd J. Normann and T. Yoshida

1. Granuloma Formation by Mycolic Acid Containing Glycolipids in Nocardia and Related Taxa — Kenji Kaneda
2. Experimental Epithelioid Cell Granulomas: Tubercle Formation and Immunological Competence — Marian J. Ridley
3. Experimental Pulmonary Foreign Body Granulomatous Inflammation and Anergy — Craig Allred
4. The Role of Interleukins in Granulomatous Inflammation and the Associated Anergy — Takeshi Yoshida
5. Monocyte-Modulating Factors in Sarcoidosis Sera — Toru Baba

6. Prominent Production of Fibronectin by Human Alveolar Macrophages in Interstitial Lung Diseases — Hiroshi Watanabe

7. Tiny Silicate Crystals Found in Macrophages of Pleural Fluid of Asbestos-Exposed Patients — Yuji Kimura

8. The Effect of Endotoxin and Gadolinium Chloride on the Acute, Septic Peritonitis in Rats — G. Lazar

Symposium 6: **September 4 (Tue)**
Room B 14:00 – 17:00

Cell-Cell Interactions in Regulation of the Immune Response

Chairpersons: Michael Feldman and T. Yamashita

1. The Accessory Cell Function of Human Alveolar Macrophages in T Lymphocyte Proliferative Responses — Morio Ohtsuka

2. I-A$^+$ Positive Macrophage Cell Lines with APC Activity — Toshinori Soejima

3. Enhancement of Monocyte Accessory Cell Function by Interferon-γ — Susanne Becker

4. Immunological Activity of a Murine Macrophage Cell Line, Immunological and Biochemical Characteristics of the T Cell Activating Factor (s) — Osami Daimaru

5. Functional Properties of Cultured Murine Thymic Macrophages, Release of IL-1 and Induction of MHC Restricted Proliferation of (T-G)-A-L Specific T Cell Line — Ruth Gallily

6. Dysfunction of Ia-Positive Antigen-Presenting Cells in Tumor-Bearing Hosts — Uki Yamashita

7. I-J Positive Lined Macrophages Replace the Splenic Accessory Cells in the Induction of Suppressor T Cells — Reiko M. Nakamura

8. Suppressor Cells Including Plastic Dish Adherent Cells in Murine Bone Marrow Chimeras — Masahiro Imamura

Symposium 7: **September 4 (Tue)**
Room D (14:00 — 17:00)

NK Cells

Chairpersons: Hillel S. Koren and S. Habu

1. Changes in Natural Killer Activities in Experimental Secondary Amyloidosis — Kouichi Kimura
2. Natural Killer Cell Activity and Tissue Distribution in Malignant Lymphoma — Masaru Nishikori
3. NK Activity, Production of Alpha-Interferon and Production of Interleukin 2 in Patients with Preleukemia — Mihiro Okabe
4. Selective Activation of Natural Killer (NK) Cell-Mediated Cytotoxicity *Induced by* Sodium Periodate Treated OK-432 — Yoshihiro Hashimoto
5. Newly Produced Small Bone Marrow Lymphocytes Bind to NK Targets — S.B. Pollack
6. A Target Cell Line for Non-Natural Killer Spontaneous Cytotoxic Cells — K. Akamatsu
7. Natural Killing of Human Blood Monocytes: Release of Monocyte Cytotoxic Factors (MCF) During Interaction with Target Cells — Atsushi Uchida

Symposium 8: **September 4 (Tue)**
Room C (14:00 — 17:00)

The Ontogeny, Phylogeny and Structure of Elements of the Mononuclear Phagocyte System

Chairpersons: Ronald B. Herberman and K. Watanabe

1. Ultrastructure and Cytochemistry of Primitive Macrophages in Human York Sacs — H. Enzan
2. Ontogeny of Macrophage Colony-Forming Cells (M-CFC) — Thomas J. MacVittie
3. An Experimental Study of the Origin of Brain Macrophages — Nam Poo Kang

4 Distribution of Anomalous Lysosomes in Monocytes and Tissue Macrophages of Beige Mouse — Yutaka Kawakami

5 Importance of the Sinusoidal Fenestration for Blood Monocytes to Settle on the Sinusoidal Surface — T. Madarame

6 Ultrastructural Analysis of Relationship Between HEV Cells and Dendritic Reticulum Cells in Germinal Centers of Human Lymph Nodes — Fumiaki Yuda

7 Langerhans Type Dendritic Cells in the Lymphnodes of Nude Mice — Hirotsugu Uda

8 Immunohistochemical Study of Dendritic Reticulum Cell in Lymph Follicle of Thyroid — Mitsunori Yamakawa

Poster Session II — **Septemer 4 (Tue)**
Room E 17:00 — 18:00

Charpersons: Sang Ho Kim, K. Harigaya and Pierre Jacques

1 Development of Splenic Ellipsoid and Its Cellular Constitution in Chick Embryo — Junpei Asai

2 Chemiluminescence Response of Functionally Different Human Peripheral Blood Mono[illegible]tes and Its Modulation by Prostaglandins — Carl G. Hedor

3 The Induction of Cytostatic Macrophages and Anti-Tumor Effects by Inflammatory Neutrophils — Alan Lichtenstein

4 Granulocyte-Macrophage Progenitor Cells in the Liver of Human Embryos — Yoshihisa Ohmishi

5 Ultrastructural Feature of the Lysozyme-Containing Cells of the Rat — Hideo Sakuma

6 Hyalocyte: A Possible Cell that Belongs to Mononuclear Phagocyte System — Yoshitsugu Tagawa

7 Development and Maturation of Fetal Rat Macrophages in Ontogenesis — Kiyoshi Takahashi

Plenary Session III: **September 5 (Wed)**
Room A+B (9:00 – 12:00)

Macrophages as Regulators of Multiple Host Systems

Presiding: Ralph van Furth and Kazuhisa Sato

1. Macrophages as Autoregulators of Mononuclear Cell Proliferation — Ralph van Furth
2. Macrophages as Regulators of the Immune Response — Howard M. Grey
3. Mode of Antigen Presentation in Association with Macrophage Ia Molecules for T Cell Recognition — Takushi Tadakuma
4. Macrophages as Regulators of the Coagulation System — Thomas S. Edgington
5. Macrophages as Regulators of the Acute Inflammatory Response — William Scott

Wednesday Symposium I **September 5 (Wed)**
Room A+B (13:00 – 15:00)

Analysis of Malignant Lymphomas with Monoclonal Antibodies

Chairperson: K. Kimura

1. Monoclonal Antibodies for the Analysis of Non-Hodgkin and Hodgkin's Lymphomas — Harald Stein
2. B-Cell Lymphomas and Their Monoclonal Antibodies — Kokichi Kikuchi
3. Immunopathological Study of T Cell Malignancies with Monoclonal Antibodies against T Cell Leukaemia Associated Antigens — Ryuzo Ueda
4. Monoclonal Antibody Study in Lymphoid Malignancy — Masanori Shimoyama

Wednesday Symposium II: **September 5 (Wed)**
Room A+B (15:10 – 17:00)

Proliferative Disorders of Langerhans and Related Cells

Chairpersons: Christian Nezelof and A. Mikata

1	Pathology of Histiocytosis-X	Christian Nezelof
2	The Role of Langerhans Cells in the Immune Response	Josef S. Smolen
3	Malignant Histiocytosis and T-Zone Histiocyte	Shaw Watanabe
4	Immunohistochemical Study of Histiocytosis-X	Yutaka Imai

Plenary Session IV: **September 6 (Thu)**
Room A+B (9:00 – 12:00)

Macrophages and Activation

Presiding: Dolph O. Adams and Tohru Tokunaga

1 Mechanisms of Target Recognition and Destruction by Macrophages — Dolph O. Adams

2 Definition of Macrophages in Various Stage of Activation by Monoclonal Antibodies — Tadayoshi Tamiyama

3 Induction of Activation in Human Monocytes by Gamma Interferon — Carl F. Nathan

4 The Molecular Basis of the Action of MAF/Interferon — Robert Schreiber

5 Macrophage Activation for Destruction of Parasites — Monte S. Meltzer

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Interrelationships Between Tumors and Mononuclear Phagocytes

Chairpersons: Hilary Koprowski and E. Tsuboi

1 Changes in the Macrophage Density in Growing Metastases — Peter J. Bugelski

2 Role of Spleen Cells Responsible for the Regulation of Cancer Metastasis — Masato Yagi

3 Functions of Macrophage in Cancer Patients — You-Hui Zhang

4 Splenic Suppressor Macrophages in Tumor-Bearing Mice — Takashi Fuji

5 Natural Cytotoxicity of Blood Monocytes in Cancer Patients — Etsuro Yanagawa

6 Human Alveolar Macrophage-Mediated Tumor Cell Killing: Production of Tumor Cytotoxic Factor(s) and Its Action — Saburo Sone

7 Antigenic and Amino Acid Sequence Homology Between HTLV and the Retrovirus Envelope Protein p15E — George J. Cianciolo

Symposium 10 — **September 6 (Thu)**
Room B 14:00 – 17:00

The Role of Mononuclear Phagocytes in Disease

Chairpersons: David S. Nelson and K. Takahashi

1 Macrophages and Tumour Biology — D.S. Nelson

2 The Origin of Gaucher Cells and Ultrastructural Composition of Their Stored Material — Makoto Saito

3 Characterization of Foam Cells and Participation of Macrophages in Atherogenesis — Kouichi Tomita

4 Alveolar Macrophage Activation of Patients with Interstitial Lung Diseases — Akihiko Sagai

5 Superoxide Production of Monocyte Derived Macrophage from Collagen Diseases — Eietsu Ouchi

6 Dysfunction of HLA-DR Positive Monocytes in SLE Patients — Fumihiko Shirakawa

7 Impaired Adherent Cell Function in Sodium Periodate ($NaIO_4$) Activation of Mononuclear Cells (MN) from Patients with Systemic Lupus Erythematosus (SLE) — R. Lemmitzer

8 Production of Fibronectin by Monocytes and Alveolar Macrophages in Patients with Progressive Systemic Sclerosis — Ichiro Kono

9 The Effects of Immuno Adjuvants on Plasma Fibronectin — Takao Kikuchi

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Room C (14:00 – 17:00)

Biology of the Neutrophil

Chairpersons: Richard B. Johnston and M. Yoshinaga

1. Production of the Lymphocyte Stimulating Factor by Polymorphonuclear Leukocytes — Tomimasa Goto
2. Properties of IgA in Polymorphonuclear Leukocytes — Zina Moldoveanu
3. Alterations in Granulocyte (G) Function with Citrate Soluble (C-S) and Insoluble (C-I) Nephropathic Immune Complexes (IC) — Edward J. Ruley
4. Phagocytosis Stimulatory Substances Released from Platelets — Haruhiko Sakamoto
5. Suppressive Effects of Nicotine on the Defense Function of Human Polymorphonuclear Leukocytes in Vitro — Sumiko Sasagawa
6. Regulation of Contractile Activity of Contractile Protein from Neutrophils — Nobuhiko Shibata

Symposium 12: **September 6 (Thu)**
Room D (14:00 – 17:00)

Immunopharmacology and Immunotoxicology of the Mononuclear Phagocyte System

Chairpersons: Jack H. Dean and I. Azuma

1. Macrophage Activation by Fatty Acid Derivatives of Glucosamine 1-Phosphate, Analogs of the Reducing-End Subunit of Lipid A Found in Escherichia coli — Masahiro Nishijima
2. Tumoricidal Capacity of Artificially Activated Murine Macrophages — Wilveria B. Atkinson
3. Shizophillan (SPG)-Treated Macrophages and Anti-Tumor Activities against Syngeneic and Allogeneic Tumor Cells I. Characteristics of SPG-Treated Macrophages — Isamu Sugawara

4 Inhibition of Tumor Metastasis with Activation of Macrophages by BRM
Takashi Yamashita

5 Antimicrobial Activity of Tuftsin, an Immunomodulating Peptide Hormone
Kenji Nishioka

6 Detection of an Alpha Interferon Messenger RNA Associated with Intracytoplasmic Alpha Interferon Activity in Activated Human Monocytes Henry C. Stevenson

7 Myelotoxicity in Mice Administered Diphenylhydantoin M. F. Luster

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Chairpersons: Sarot Chandrasekhr, H. Hara and Peter Abramoff

1 Dermatopathic Lymphadenopathy Shigeyuki Asano

2 Induction of Tumoricidal Macrophages and Granulocytes by the Intranasal Application of MIP PL, a Lipophylic Muramyl Peptide D.G. Braun

3 Altered Cellular Mechanisms of Tumor Resistance Following Exposure to Carcinogenic Polycyclic Aromatic Hydrocarbons (PAH) Jack H. Dean

4 Lymphoreticular Cells Endotoxin (LPS) and D-Galactosamine (D-GAL) Induced Liver Injury J. Freter

5 Cellular Responses to Lipopolysaccharide in the Mouse Spleen H. Hara

6 Effects of Estrogen on RES with Special Reference to Hemopoiesis
Takeshi Hayama

7 Effect of Yoshida Sarcoma on the Sanarelli-Shwartzman Reaction Induced by Liquoid
Elizabeth Husztik

8 Augmentation Effect of Murine Interferon-α, β on Hydroxyl Radical Production in Murine Macrophages M. Ito

9 Morphological Changes of Human Macrophages in Patients with Ovarian Carcinoma and Its Characteristics Minoru Kaneko

10 Alcide, an Antimicrobial that Controls Wound Fibroplasia Alan J. Kenyon

Sept. 6 (Thu)

11 Immunopathological Study on Myoglobin Positive and Anti-Myoglobin Antibody Positive Cells in Myasthenic and Non-Myasthenic Thymuses — Takane Koeda

12 Effect of Passively Transferred Macrophages on Metastatic Spread of Hamster Lymphoma — Harukazu Mashiba

13 Tumor Inhibitory Effect of Intralesional Injection of Bradykinin and Immunostimulants in Mice — Keiko Matsunaga

14 Recognition of Foreignness by Phagocytes as Observed by Their Response to Biological Response Modifiers — Kaoru Morikawa

15 Antitumor Activity of Newborn Mouse Macrophages — Shigeru Muramatsu

16 Potentiation of Tumoricidal Activity in Human Mohocytes by Muramyl Dipeptide and Its Lipophilic Analog Entrapped in Liposomes — Seiji Matsumura

17 A Case of Multiple Myeloma with Hemophagocytosis — Masaki Nakazawa

18 Presentation of Amyloid Forming Cell Lymphocyte Interactions Found in the Spleen and Liver from H-NTA-Induced 'I' Amyloidosis' Mice — Motohiro Ogura

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20 *Glucan Therapy Enhances Hemopoietic Repopulation, Inhibits Sepsis and Enhances* Survival in Irradiated Mice — Myra L. Patchen

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22 Activation of Phagocytes by Acidic Mannan from Bakers' Yeast — Shigeo Suzuki

23 Kimura's Disease (Eosinophilic Lymphfolliculoid Granuloma) — Keizo Takaki

24 Effect of Isoprinosine (ISO) on the Interleukin-1 Production in Vitro in Patients with Acquired Immunodeficiency Syndrome (AIDS) — Kwong-Y. Tsang

25 Atypical Letterer-Siwe Disease with Marked Erythrophagocytosis — Yukiko Tsunematsu

26 Macrophage-Mediated Indirect Effect of *Interferons* on the In Vivo Tumor Cell Growth — Kazuko Uno

27 Ultrastructure of Cordal Macrophages in Spleens from Patients with Idiopathic Thrombocytopenic Purpura — Yoshimi Yamashita

Plenary Session V: **September 7 (Fri)**
Room: A+B (9:00 – 12:00)

Macrophages and Stimulus-Response Coupling

Presiding: Ralph Snyderman and Kaoru Onoue

1 Transduction Mechanisms of Chemo-Attractant Receptors — Ralph Snyderman

2 The Role of Protein Kinase C in Stimulus Response Coupling — Kozo Kaibuchi

3 The Motor of Leukocytes — Wartwig

4 Chemotaxis of Macrophages — Hideo Hayashi

5 Transduction Mechanisms of Fc Receptors — Jay C. Unkeless

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Macrophages and Regulation of the Immune Response

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2 Early Cellular Responses to Concanavalin A in the Mouse Spleen — Kensuke Matsusaki

3 Suppressed Lymphocyte Production by a Transplanted Granulocytosis Inducing Mammary Carcinoma in Mice — M.Y. Lee

4 Xenogeneic Cell Interaction between Antigen-Specific Murine T Cells and Human Antigen Presenting Cells — Koji Yabu

5 Fcγ Receptor-Mediated Regulation of B Lymphocyte Response to Antigen — Mariano F. La Via

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Cell Lines, Markers and Differentiation of the Mononuclear Phagocyte System

Chairpersons: William S. Walker and W. TH. Daems

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2 Expression of 5' Nucleotidase Activity and Wheat Germ Agglutinin Binding in Mononuclear Phagocytes from Bone Marrow Cultures — I. A. Cansed

3 Isolation of Functionally Distinct Rat Macrophage Subpopulations by Percoll Density Gradients and Centrifugal Elutriation — Robert H.J. Beelen

4 Characterization of Cell Lines Derived from Adult T Cell Leukemia and Lymphoma (ATL) — Takayuki Harada

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6 Establishment of Human Monocyte Cell Lines and Secretion of Interleukin 1 — Abraham J. Treves

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Chemotaxis and Accumulation of Elements of the Mononuclear Phagocyte System

Chairpersons: George J. Giancotto and T. Kambara

1 Human Monocyte Chemotaxis: 3 Populations Distinguished by Functional and Flow Cytometric Analysis — Edward J. Leonard

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3 A Chemotactic Factor for Macrophages Produced in Vivo — Tetsu Kawaguchi

4 The Effect of LTB$_4$ on Monocyte Chemotaxis — Masako Katoh

5 Effect of fMet-Leu-Phe and Autologous Plasma on Adhesion of Human Polymorphonuclear Leukocytes — Tatsuchiro Sakatani

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Norio Asou

2 Antisera against the Inducer for the Differentiation of Human Leukemic Cells to Monocyte Macrophages
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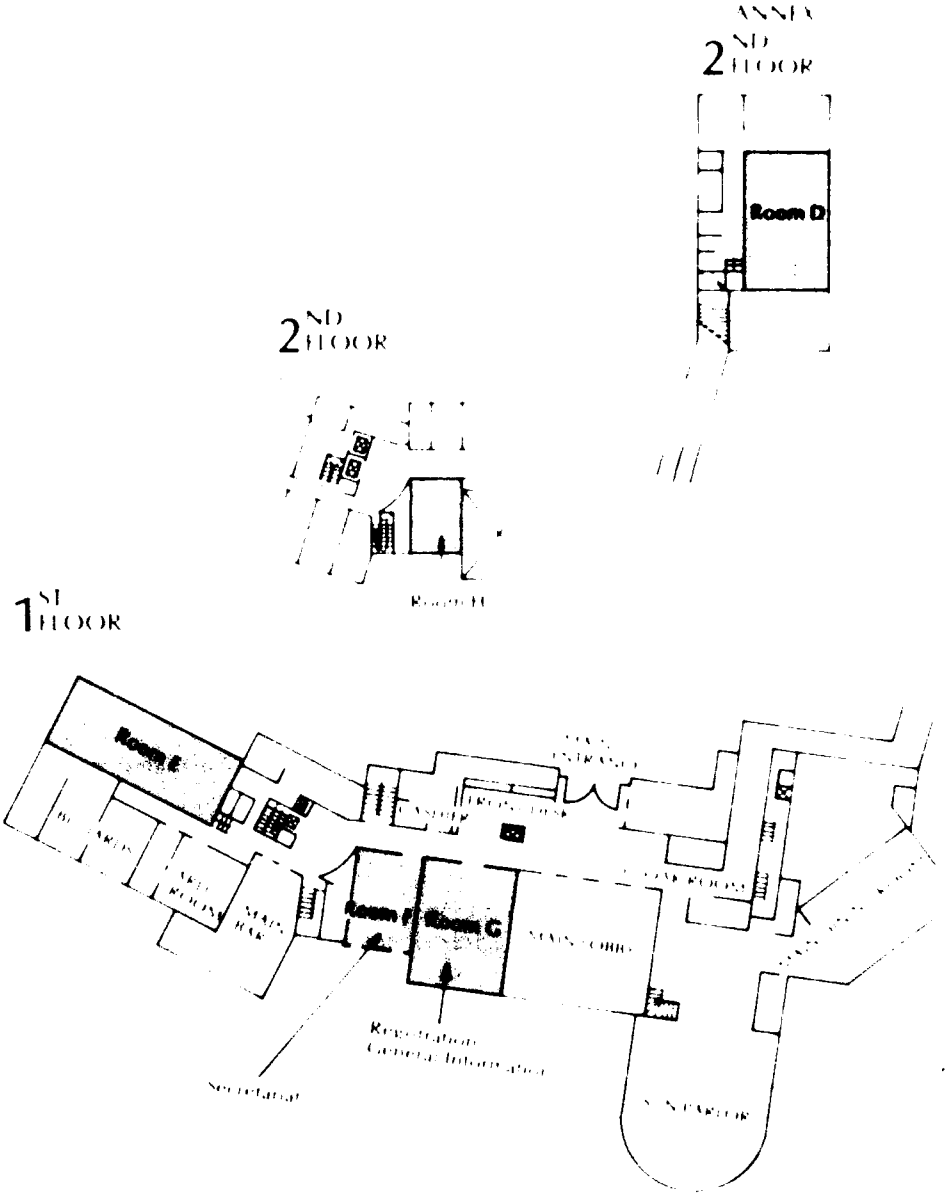
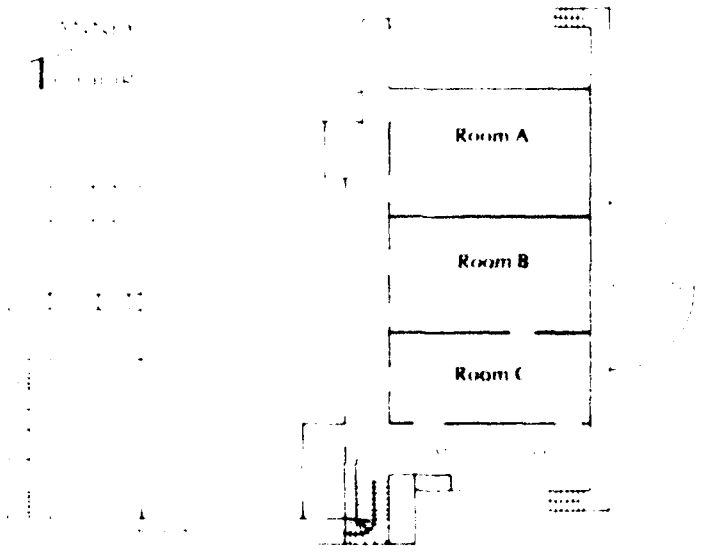
25 An Electronmicroscopic and Karyometric Study on Non-Hodgkin's Lymphoma with Special Reference to Nuclear Irregularity — Yoshiyuki Uyesaka

CONGRESS ROOMS

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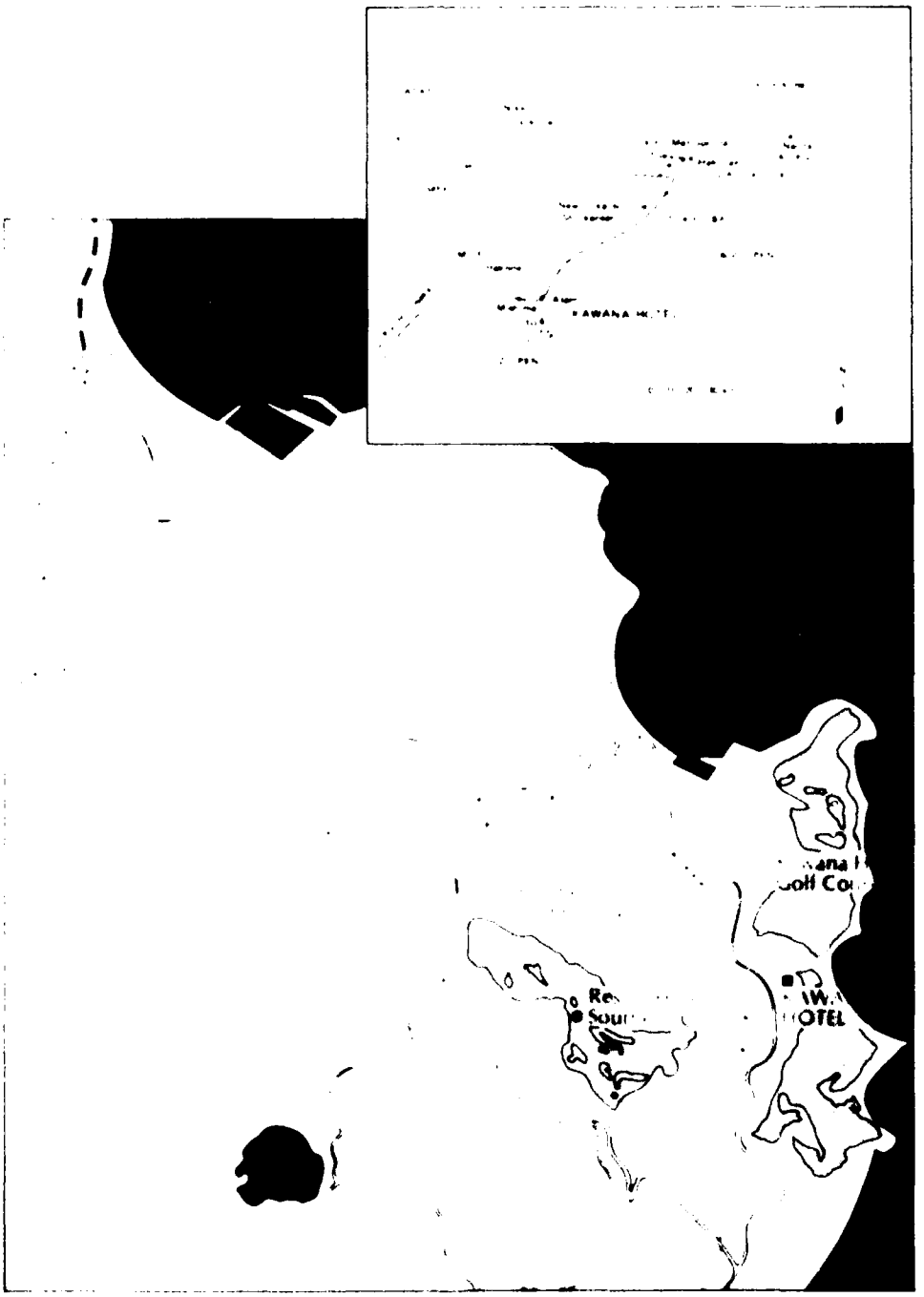
MAP OF ITO-KAWANA



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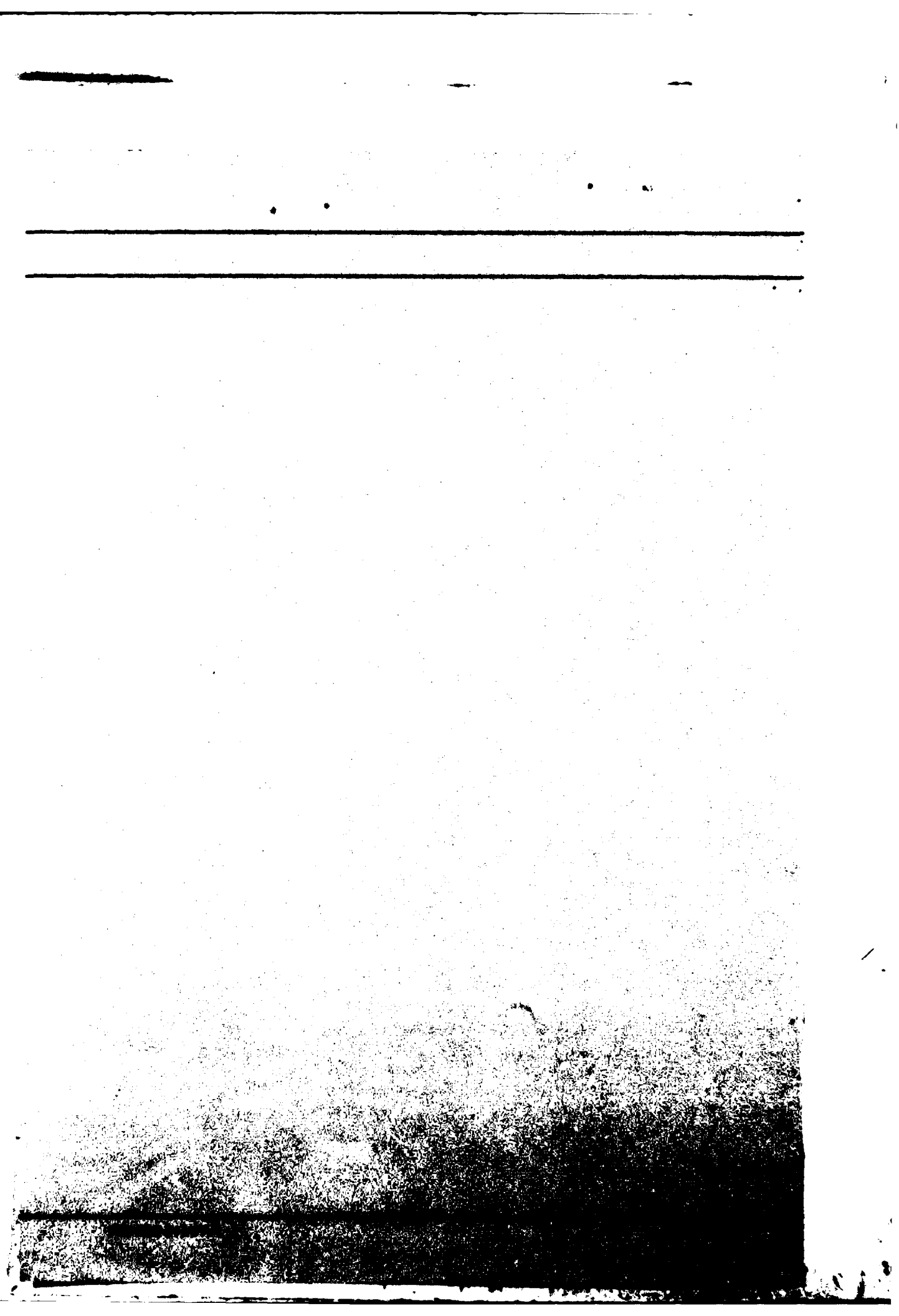

10th INTERNATIONAL RES CONGRESS

Ito, Japan

September 2-7, 1984

ABSTRACTS

10th INTERNATIONAL RES CONGRESS

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ABSTRACTS

This Congress is supported by the grants from the Commemorative Association for the Japan World Exposition (1970).

CONTENTS

Monday, September 3

S1-1

THE EFFECTS OF THE VARIOUS AGENTS ON THE CULTURED KUPFFER CELLS

[illegible], KAZUNORI NOGUCHI, TOSHIHIRO KAWAHARA, HIROSHI ABE, KYOICHI TANIKAWA
THE FIRST INTERNAL MEDICINE, KURUME UNIVERSITY SCHOOL OF MEDICINE, KURUME, JAPAN

Materials and methods
The isolated rat Kupffer cells prepared by pronase digestion and centrifugation with the density gradients were cultured for 24 hours in vitro. The cultured Kupffer cells were incubated in the medium containing cytochalasin B, colchicine, streptococcal preparation OK-432, β-1,3Glucan (SPG), and ethanol. The endocytic function was examined with the formalin-fixed rat erythrocytes, latex particles (5.4um, 2.2um, in diameter) and radioactive colloidal particles (^{125}I-PVP). The effects of the various agents on the endocytosis of the cultured Kupffer cells were determined by the light & electron microscopies and the radioactivity measurement.
Results
The uptake of formalin-fixed rat RBC or latex particles of 5.4um into the Kupffer cells was inhibited by colchicine or cytochalasin B treatment which reduced the number of the pseudopodia of the Kupffer cells. OK-432 or SPG treatment which increased the number of the pseudopodia of the Kupffer cells stimulated the uptake of ^{125}I-PVP. Ethanol reduced the uptake of latex particles of 2.2um.
Conclusions
While ethanol and the agents which inhibit the function of the cytoskeletons in the Kupffer cells reduced the endocytosis of the foreign materials, the immune-stimulators increased the endocytosis of them.

S1-2

PHENOTYPIC CHARACTERIZATION OF GAMMA INTERFERON-INDUCED HUMAN MONOCYTE POLYKARYONS (MP). J.B. WEINBERG, M.A. MISUKONIS, M.M. HOBBS. VA and Duke, Durham, NC 27705.

We have previously demonstrated that highly purified recombinant human gamma interferon (IFN-γ) causes normal human peripheral blood monocytes to fuse and form MP. These MP, which resemble those seen in vivo in patients with granulomatous diseases, formed over a 36 to 72 hour period in cultures with 10% autologous, untreated serum. The MP were 28 to 1000 microns in diameter and contained 2 to 150 nuclei/MP with a fusion index of 40 to 70%. The peak effects were seen at doses of 20 to 100 units/ml (0.1 to 0.5nM). The IFN-γ effect was abolished by treatment at 56 C for 4 hours, pH 2 for 3 hours, or with mouse monoclonal anti IFN-γ antibody. As determined by autoradiography, the MP did not incorporate tritiated thymidine into their nuclei. The MP contained nonspecific esterase and tartrate-resistant acid phosphatase. Various preparations of recombinant and natural alpha and beta interferons did not cause the MP formation. Populations of IFN-γ-treated monocytes had increased levels of acid phosphatase, plasminogen activator, and H_2O_2 production in response to phorbol myristate acetate. However, when assessed on an individual cell basis, the MP reduced little or no NBT, while the uninuclear monocytes reduced large amounts. The MP phagocytized latex spheres normally, but there was diminished phagocytosis of antibody-coated sheep erythrocytes. The uninuclear monocytes contained antigens recognized by the monoclonal antibodies LeuM3 (antimonocyte), 9E1 (anti HL-60), lysozyme, and TE5 (thymic macrophage). The MP had normal lysozyme, but there was no or very little LeuM3, 9E1, and TE5 in these MP. Thus, IFN-γ induces MP formation by fusion of blood monocytes, and the MP are phenotypically different than the monocytes.

S1-3

[illegible] EXPRESSION OF LPS RECEPTOR ON MOUSE LUNG MACROPHAGES BY LYMPHOKINE [illegible]
[illegible] A[illegible]WA, [illegible] TOKUNAGA. Dept. of Tuberculosis, National Institute of Health, [illegible], Japan.

While peritoneal resident macrophages (PRM) or peptone-induced peritoneal [illegible] (PEM) preincubated with bacterial lipopolysaccharide (LPS) showed [illegible] against [illegible]Cr labeled [illegible] cells, lung macrophages (LM) did not respond to LPS. Treatment of LM with indomethacin ([illegible] M) did not influence on the [illegible]responsiveness of LM. LM obtained from BCG-infected mice or LM treated in vitro with [illegible] culture supernatant of normal spleen cells incubated with [illegible] ug/ml Con A [illegible] at 37 C for [illegible] days could respond to LPS, and became cytotoxic. The active [illegible] in the lymphokines (LK)-rich supernatant was tried to separate by sephacryl [illegible] chromatography, and they were distributed in a broad range of molecular weights from about [illegible] to [illegible], corresponding to the fractions possessing [illegible] to activate PEM. LM were insensitive to direct toxicity of LPS, while PRM [illegible] were killed by LPS. About [illegible] of PRM and PEM were stained with FITC-LPS, while less than [illegible] of LM were stained. However, about [illegible] of LM treated with LK in vitro or LM obtained from BCG-infected mice could bind FITC-LPS. These results [illegible] that the unresponsiveness of LM to activation for tumor cytotoxicity by LPS [illegible] attributed to the lack or very low expression of LPS receptor on their cell [illegible], and that LM treated with LK in vitro or LM from BCG-infected mice express [illegible], which [illegible] LM to respond to LPS and become cytotoxic against tumor [illegible].

S1-4

[illegible]
[illegible] AMA[illegible], [illegible] KAWA, [illegible] AKAMA[illegible] [illegible], Japan.

[illegible]

S1-5

SELECTIVE TUMOR CELL LYSIS BY NON-SPECIFICALLY ACTIVATED MACROPHAGES DERIVED FROM LONG-TERM BONE MARROW CULTURES. J. LOEWENSTEIN, R. GALILY. The Lautenberg Center for General and Tumor Immunology, The Hebrew University-Hadassah Medical School, Jerusalem, Israel.

It has been well documented that most neoplastic cells are lysed by non-specifically activated macrophages. Resident or elicited peritoneal macrophages are most commonly applied, however, these cell populations are heterogeneous and may display lysis in absence of activating agents. Pure macrophage populations with potential cytolytic activity may be obtained from 1-2 week-old in vitro cultures of murine bone marrow (BM) explants. We investigated the capability of macrophages derived from long-term BM cell cultures to lyse ^{3}H-TdR prelabeled murine target cells, comparing the results to those yielded by thioglycollate-elicited peritoneal macrophages and 1-2 week-old BM-derived macrophages. At all stages of long-term development, BM-derived macrophages had to be activated by LPS, Con A-induced lymphokines, M. orale or synergistically acting combinations of these agents, to lyse Ag fibrosarcoma cells. Optimal cytolysis was observed 72 hr after initiation of the experiment at E:T ratios of 10:1 and higher with macrophages present in a monolayer. The selectivity of killing various target cells was similar for different types of macrophages. Thus, all macrophages did lyse most tumor cells, but not normal fibroblasts. None of the macrophage populations could kill the M109 adenocarcinoma, whereas the B16 melanoma was lysed by BM-derived macrophages only. Our results demonstrate that macrophages derived from long-term BM cell cultures are a reliable source of effector cells in the study of macrophage-mediated non-specific tumor cell lysis.

S1-6

Fc RECEPTOR MODULATION AND CYTOTOXIC ACTIVITY OF PORCINE PULMONARY ALVEOLAR MACROPHAGES. [illegible] and Robert Rothlein. University of Health Sciences/The Chicago Medical School, North Chicago, IL 60064.

Mechanisms of Fc receptor (FcR)-dependent activation of porcine pulmonary alveolar macrophages (PAM) for cytotoxicity has been investigated. It was found that PAM, which were exposed to immobilized immune complexes (IIC) or immune complexes (IC) in suspension in conjunction with cytochalasin B, became nonspecifically cytotoxic to tumor and autologous red blood cell targets in an ^{51}Cr-release assay; whereas PAM that were exposed to only IC in suspension were not nonspecifically cytotoxic. Furthermore, it was found that PAM were not able to internalize their IC-bound FcR when the IC were immobilized or when cytochalasin B was present in the assay. Also, we found that the lytic mechanism involved in the nonspecific cytotoxicity generated by IIC or IC in suspension in conjunction with cytochalasin B was peroxide-dependent whereas the lytic mechanism in conventional antibody-dependent cellular cytotoxicity by PAM was peroxide-independent. In addition, it was found that PAM exposed to IIC secrete more prostaglandin E (PGE) than PAM exposed to IC in suspension. Furthermore, it was found that preculturing PAM with indomethacin at doses which inhibited all PGE secretion, had no inhibitory effect on IIC-dependent nonspecific cytotoxicity, while hydrocortisone, which was much less potent inhibitor of PGE secretion, greatly inhibited IIC-dependent nonspecific cytotoxicity. These data indicate that PGE secretion and cytotoxicity mediated by modulation of FcR are independent functions of PAM. It is possible that either one or both of these macrophage functions contributes to the pathogenesis of tissue injuries in some inflammatory auto-immune diseases.

Supported in part by USPHS Grant CA-38354

S1-7

[illegible] STIMULATE PHAGOCYTIC SUPEROXIDE ANION GENERATION IN [illegible] IN THE ABSENCE OF COMPLEMENT ACTIVATION IN VITRO. T.J. HOLZER, L. [illegible] MAUL, P.E. NELSON, AND B.R. ANDERSEN. Department of [illegible] Medical Center, [illegible]

M. leprae is an intracellular pathogen that is expected to activate and proliferate within the monocyte-macrophage (MO) series in leprosy. M. leprae is apparent [illegible] M. A rodent model of leprosy caused by M. lepraemurium (MLM) is also [illegible] The mechanisms by which intracellular pathogens resist destruction are not [illegible] but may involve resistance to toxic oxygen derivatives and [illegible] Rats and animals with lepros, appear to have normal phagocytic cell [illegible] we have reported that M. leprae failed to stimulate murine MO HMPS activity [illegible] and chemiluminescence. In addition, M. leprae and MLM did not stimulate [illegible] neutrophil, monocyte or murine MO superoxide anion (O_2^-) generation at bacteria to cell ratios up to 100:1. In contrast, M. bovis BCG at 10:1 stimulated all [illegible] to release significant amounts of O_2^-. M. [illegible] at 5:1 ratios [illegible] clinical isolate of M. tuberculosis also failed to stimulate O_2^- release. At ratios greater than 50:1, MLM and M. leprae incubated [illegible] normal human serum (NHS), washed [illegible] elicited phagocytes caused a 50% or greater O_2^- release. Recently, C5 conversion [illegible] have shown that M. leprae, MLM, and M. [illegible] strains, Kansasii, and tuberculosis fail to activate complement (C5) in NHS or leprosy patient sera. BCG, however, [illegible] of C5 at $\times 10^8$/ml in NHS and greater than 90% in patient sera. Serum treatment of BCG enhanced O_2^- release by all three cell types. Only BCG, of the mycobacteria tested, appears to activate C and to stimulate phagocyte O_2^- generation.

S1-8

THE REGULATORY ROLE OF LIPOXYGENASE PRODUCTS IN THE STIMULATED STATE OF RAT KUPFFER CELLS. M. BIRMELIN, K. DECKER. Biochemisches Institut, Universität Freiburg, Hermann-Herder-Str. 7, D-7800 Freiburg, Federal Republic of Germany.

Primary cultures of rat Kupffer cells released besides other cyclooxygenase products mainly prostaglandin E_2 (PGE_2) up to 18 ng/10^6 cells after 24 h challenge with lipopolysaccharide (LPS) or 30 min stimulation with phagocytosable material, e.g. zymosan. After phagocytosis arachidonic acid metabolites of the lipoxygenase pathway were also detected by HPLC and HPTLC, thereby mainly leukotriene C_4 (LTC_4). In the presence of 10 μM of the lipoxygenase inhibitor nordihydroguaiaretic acid (NDGA) the LPS- and zymosan-stimulated PGE_2 release was abolished. When the liver cell plates were incubated with the leukotriene receptor antagonist FPL 55712, the zymosan provoked PGE_2 synthesis was depressed to 2.5 ng/10^6 cells by 0.5 μM of the antagonist, while O_2^- production, measured by chemiluminescence, was completely inhibited by 1.5 μM FPL. During phagocytosis in the presence of 10 μM NDGA a 35% inhibition of chemiluminescence was observed. Unexpectedly, when exogenous LTA_4, LTC_4 or LTB_4 was added to the Kupffer cells no stimulatory effect on PGE_2 release was found, also the zymosan-induced PGE_2 synthesis was not influenced by these mediators of inflammation. The simultaneous addition of 1 nM LTB_4 to phagocytosing Kupffer cells, however, decreased the stimulated PGE_2 production significantly. A regulatory role of lipoxygenase products on the activated state of liver macrophages is concluded.

S2-1

DISAPPEARANCE AND REAPPEARANCE OF RESIDENT MACROPHAGES: IMPORTANCE IN C. PARVUM INDUCED TUMORICIDAL ACTIVITY. S. HASKILL, S. BECKER. University of North Carolina, Chapel Hill, NC 27514.

We have used flow cytometry to investigate the in vivo contribution of resident macrophages in the response to C. Parvum. Macrophages were labelled in situ with blue fluorescent covaspheres 72 hours prior to stimulation with FITC conjugated C. Parvum. Resident macrophages disappeared within 5 hours of administration of the bacteria. At 24 hours, fluorescent fibrinous adhesions were observed at numerous sites in the peritoneum, these contained macrophages which were now larger in size than resident cells and contained both blue spheres and fluorescent bacteria. In addition, there were numerous bacteria-containing granulocytes. The resident cells associated with large numbers of bacteria and levels of beads similar to control animals did not reappear in significant numbers until 72 hours. C. Parvum induced cytotoxicity was modestly enhanced in the macrophages which had also received spheres, but control macrophages given only spheres were not cytotoxic. Flow cytometric analysis of the fibrinolytic potential of the reemerging cells indicated that plasminogen activator-like activity was markedly elevated. Thus, resident cells disappear apparently in a coagulation dependent reaction and reappear as cytotoxic macrophages when fibrinolytic activity develops sufficiently to permit their emergence from the fibrinous adhesions.

S2-2

ENDOTOXIN INDUCED MONOCYTE/MACROPHAGE PROCOAGULANT ACTIVITY IN THE RAT REQUIRES COLLABORATING T-LYMPHOCYTES. Peter A. Lando and Thomas S. Edgington. Department of Immunology, Research Institute of Scripps Clinic, La Jolla, CA 92037.

The lymphoid system of a number of species responds to bacterial endotoxin (LPS) wherein cells of the monocyte/macrophage lineage are rapidly induced via collaborative T-lymphocytes to initiate the extrinsic coagulation protease pathway. It has been claimed that this response, basic to the Schwartzmann reaction, is lacking in rats. We have examined this in Fischer 344, BN and Lewis rats. When peripheral blood mononuclear cells (PBM) were stimulated in vitro with LPS a rapid (4 hours) procoagulant (PCA) response was observed, as based on acceleration of clotting of recalcified human or rat platelet poor plasma. PCA was not physically dissociated from viable PBM by 5mM EDTA, consistent with an intrinsic plasma membrane initiator molecule rather than calcium bound gamma carboxylated glutamic acid (GLA) containing proteases. The induction of monocyte PCA was prevented by cycloheximide and actinomycin D implicating new gene transcription and protein biosynthesis. Cultivation of PBM with warfarin or vitamin K did not affect the endotoxin induced PCA, indicating the activity not to be attributable to GLA containing proteins. No inhibition of cellular PCA was produced by serine protease inhibitors, but with $HgCl_2$, a cystein protease inhibitor, the PCA was abolished. The induced rat PCA was dependent on Factor X since inhibition of the PCA was produced by a rabbit anti-rat Factor X antibody. Isolation of monocytes and T-lymphocytes from LPS stimulated PBM revealed the PCA to be expressed by monocyte populations. When isolated rat T-lymphocytes and monocytes were separately exposed to LPS, PCA was not induced. When the cells were combined, however, LPS induced PCA was observed, consistent with a requirement for cellular collaboration between T-lymphocytes and monocytes in this response. Supported by NCI grant CA-28166.

S2-3

S2-4

S2-5

GM-CSA PRODUCTION BY HUMAN MONOCYTE SUBSETS. J.R. ZUCALI, M.A.GROSS, R.S. WEINER. University of Florida, Gainesville, FL 32605.

Human peripheral blood monocytes (PBM) have been implicated in a variety of immunological and hematopoietic responses. Using elutriation centrifugation and Percoll gradient centrifugation, we have recently obtained two purified subpopulations of PBM which differ in size. The small monocytes (modal volume 354 u^3) represent about 30% of the PBM; the larger monocyte population (modal volume 380 u^3) represent the rest. The larger monocyte population contains 90% OKM1, Leu3, esterase positive cells while the separated smaller monocyte population is made up of 60-70% OKM1, Leu3, esterase positive cells. In the present study, we compared both PMB subpopulations with unseparated monocytes for their ability to produce stimulators of granulocyte-macrophage colony forming cells (CFU-GM). Both unseparated PBM and the separated monocyte populations were capable of producing granulocyte-macrophage colony stimulating activity (GM-CSA) in a cell-dose dependent manner whether used as a conditioned medium source or as an adherent underlayer in the agar colony assay. To rule out the effect of contaminating T lymphocytes, both the stimulator populations and the target nonadherent human bone marrow cells were T-cell depleted by sheep red blood cell rosetting. The property of adherence was not essential for GM-CSA production since stimulatory activity could also be found in conditioned medium obtained from separated monocytes cultured in teflon bottles to prevent adherence. In conclusion, we have obtained two subpopulations of PBM based on size. Both populations, whether adherent or in suspension, are capable of producing GM-CSA in culture and this GM-CSA production appears to be T-cell independent.

S2-6

[illegible]

S2-7

IMMUNOHISTOCHEMICAL LOCALIZATION OF S 100 PROTEIN SUBUNITS IN THE HUMAN LYMPHORETICULAR SYSTEM. T. AKAGI, K. TAKAHASHI, Y. OHTSUKI. Department of Pathology, Kochi Medical School, Nankoku, Kochi, 781-51, Japan.

S 100 protein, which was previously thought to be restricted to nervous tissues, has been found in Langerhans cells (LC) and interdigitating reticulum cells (IDC), blood monocytes and macrophages. S 100 protein is not a single protein, but a mixture of at least three similar proteins, S 100ao, S 100a, and S 100b, with a subunit composition of $\alpha\alpha$, $\alpha\beta$, and $\beta\beta$, respectively. However, S 100ao protein is only a minor component and antisera prepared with bovine brain S 100 practically react only with S 100b and S 100a. In the present study, immunohistochemical localization of S 100 protein and S 100ao protein in human lymphoreticular system was examined by using monoclonal antibody directed against each α subunit or β subunit of S 100 protein. S 100 protein immunoreactivity was detected in LC, IDC, and histiocytic reticulum cells, but not in ordinary macrophages and blood monocytes. S 100ao protein immunoreactivity was detected in blood monocytes, macrophages of lymphnode, alveolar macrophages of lung, and small numbers of Kupffer cells of liver. S 100ao immunoreactivity was also detected in epithelioid cells, Langhans giant cells, and foreign body giant cells. The present findings suggest that the presence of S 100ao protein in the cytoplasm is one of the characteristic features of cells in human mononuclear phagocyte system. The detection of S 100b, but not S 100ao, immunoreactivity in LC and IDC also suggests that they are independent of the monocyte macrophage system. S 100 protein may be a novel cytoplasmic marker for cells of the human monocyte macrophage system.

S3-1

S3-2

[illegible], WILLEM A. [illegible] and ELISABETH C. M. HOEFSMIT
[illegible] Section Medical Faculty, Free University, NL 1007 Amsterdam
[illegible] Department of Tumor Virology, St. Jude Children's Research Hospital, Memphis, TN 38101

[illegible] the fixation method, which has an enhanced sensitivity as compared with [illegible] assays we have recently shown that in C3H mice the expression of Ia antigen is [illegible] on subpopulations of peritoneal macrophages. This also holds true for the rat system (OX2 MAb) as tested with anti Ia 17 in rosetting and functionality in antigen presentation. Moreover, commonly used fixatives as paraformaldehyde and glutaraldehyde affect dramatically the detectability of Ia antigen on a variety of cells in the rat, mouse and human system and therefore results obtained with their use must be interpreted with caution. For ultrastructural immunocytochemistry a short fixation in 0.1% paraformaldehyde resulted in preservation of Ia on only ≈ 10% of the macrophages, whereas the rosetting assay (without fixation) detected about 30% of Ia positive macrophages. However, after immunization with live BCG the immunocytochemistry method detected about 90% of Ia positive cells, while the rosetting method gave about the same percentage (≈ 90%). This means that indeed the immune status of the animal is responsible for a change in Ia expression of peritoneal macrophages (as is the addition of lymphokines in vitro), however, the detection of this observation is strongly dependent of method of assay and fixation used.

S3-3

[illegible] ON THE PRESENCE OF ANTIGENIC DETERMINANTS ON NORMAL, REACTIVE AND [illegible] [illegible] I.Kleyne and [illegible] van Unnik, Institute of Pathology, [illegible] Pasteurstraat [illegible], [illegible] UTRECHT, THE NETHERLANDS.

[illegible] examined macrophages [illegible] which are present in reactive lymph [illegible] which are present in several soft tissue sarcomas. Furthermore, [illegible] examined the malignant derivates [illegible] (malignant histiocytosis). For [illegible] the staining pattern of several monoclonal antibodies [illegible] the immunoperoxidase technique. The MA used are commer[illegible] and spleen [illegible] detected with [illegible] MA, whereas [illegible] in the paracor[illegible] with [illegible] MA. Tolilox [illegible] patterns. [illegible] MA did not stain the same cases every ti[illegible] MA stained [illegible] whereas the other three MA stained [illegible] demonstrated [illegible] nine cases [illegible] all MA [illegible]

[illegible] results we [illegible] that [illegible] few of the reactive [illegible] were stain[illegible] were stained with [illegible] MA. Further [illegible] staining pattern. [illegible] MA reacted with these cells.

S3-4

S3-5

COMPLEMENTARY ROLES OF KUPFFER CELLS (KC) AND LIVER ENDOTHELIAL CELLS (LEC) IN THE ENDOCYTIC FUNCTION OF RES IN THE LIVER. B. SMEDSRØD, H. PERTOFT, T.C. LAURENT. Department of Medical and Physiological Chemistry, University of Uppsala, Biomedical Center, Box 575, S-751 23 Uppsala, Sweden.

Cells lining the liver sinusoids constitute an important part of the total RES. KC has been considered to be the cell mainly concerned with the scavenger function but recent work suggests that the LEC exhibits a significant endocytic activity and plays an important role in the RES of the liver. In order to study the functions of each cell separately we have developed a method to isolate and culture KC, LEC and parenchymal cells (PC) from a single rat liver. The cells are dispersed by collagenase perfusion of the liver, centrifuged in Percoll^{R} and grown on different substrates, which yields cell cultures at least 90-95% pure. Endocytosis of particulate material was followed by phase contrast microscopy and internalization of labelled soluble ligands by fluorescence microscopy or uptake of radioactivity. Particulate ligands, i.e. glutaraldehyde treated erythrocytes or erythrocytes covered with IgG or C3b were phagocytosed exclusively by KC, although IgG-covered erythrocytes also were bound to the LEC surface. Soluble ligands, i.e. hyaluronic acid, chondroitin sulphate and chondroitin sulphate B (mucoitin), formylated serum albumin, ovalbumin and a tissue plasminogen activator, were internalized and degraded by LEC only. None of the ligands was taken up by PC. These observations may reflect a general principle in the allocation of functions to the sinusoidal cells, i.e. KC are responsible for the uptake of particulate ligands whereas LEC internalize soluble ligands.

S3-6

S3-7

M. Nowakowski, ... N.Y. Downstate Medical Center, Brooklyn, N.Y. 11203.

Expression of surface receptors for complement and for Fc-IgG by murine lung and peritoneal macrophages was measured following influenza A virus infection and interferon treatment in vitro. Influenza virus selectively decreased the binding of complement-coated sheep erythrocytes (EAC'K) by peritoneal macrophages (from 60% to 18% EAC'K-binding cells), but did not alter the expression of Fc-IgG receptors (75% EAIgG-binding cells). Pretreatment with murine interferon (IFN) α and β for 18 hrs blocked this virus-induced modulation of complement receptor expression in peritoneal macrophages. Lung (bronchoalveolar) macrophages from BW mice contained a very small fraction of cells with surface complement receptors (3%). This number remained low after influenza virus infection, but a striking increase (tenfold) of the number of complement receptor positive cells followed the incubation of lung macrophages in the presence of IFN. Moreover, once induced, these complement receptors were resistant to modulation by influenza virus. These results indicate that, 1. direct interaction of influenza virus with macrophages may affect specific functions selectively, and 2. IFN, in addition to its direct anti-viral effects may activate lung macrophages and alter their functions.

S4-1

ADOPTIVE TRANSFER OF IMMUNE RESPONSIVENESS FROM HEAVILY INFECTED ASIALO DONORS. [illegible] COLLINS, K.P. KEPPLER. Trudeau Institute, Saranac Lake, NY 12983

M. [illegible] kansasii induces a persistent systemic infection in intravenously [illegible] (B6D2) F_1 hybrid mice in which the normal T-cell-mediated [illegible] seem incapable of eliminating the bacterial population from the tissues. Despite this apparent lack of immunity, the spleens of heavily infected mice exhibit a marked and sustained increase in cellular proliferation and enhanced non-specific macrophage activation. The anti-Listeria activity peaks about the same time as the M. kansasii counts within the lungs and spleen pass into their prolonged plateau growth phase. Mice infected with 10^6 CFU M. kansasii possess a population of spleen T-cells capable of passively transferring protection (5 to 10-fold reductions in viable counts after a 28 day incubation period). The optimum response occurred when 8×10 [illegible] (approximately 1 spleen equivalent) were infused into sublethally irradiated (450 rad) x [illegible] recipients. The transferred T-cells activated the recipient host's own macrophages which then inhibited the further growth of the challenge organism in vivo, although they were unable to inactivate organisms already established in the tissues. When the donor mice were inoculated with large numbers of M. kansasii [illegible] CFU), the macrophages activated were able to limit the growth of the organisms within the spleen but all attempts to detect immune T-cells were unsuccessful, even when the number of cells transferred to the irradiated recipients were increased to 4 spleen equivalents. Thus, M. kansasii seems to induce the formation of activated macrophages by two separate mechanisms: the first is T-cell dependent, while the [illegible] cells in heavily infected, anergic donors is not.

S4-2

[illegible] SUPPRESSION AND RESISTANCE TO LISTERIA MONOCYTOGENES. M.L. CHEPENIK, [illegible] State University of New York at Buffalo, Buffalo, NY 14214.

Mice were immunized with 10^5, 10^6, 10^7 or 10^8 BCG by either intravenous (IV) or [illegible] (IP) route or treated (LHEP). Subcommittis groups (normal and BCG-infected mice were challenged IV or IP into the tail [illegible] with either 10^3, 10^4 or 10^5 [illegible] and viable counts of Lm were made 2 hr later. After IV immunization, resistance to 10^5 Lm IV was proportional to the immunizing dose of BCG, resulting in 2.5, 3.5 and 5.5 $\log_{10}$ units after 10^5, 10^6 and 10^8 BCG IV, respectively. Mice immunized with 10^8 BCG IV were resistant to all challenge doses of Lm. However, mice immunized with 10^6 BCG IV had high resistance, 3.5 $\log_{10}$ units, to 10^3 and 10^4 Lm, but only 0.7-1 $\log_{10}$ units of resistance to 10^5 Lm. Mice immunized with 10^5 BCG IV were no more resistant to 10^5 Lm than normal controls. Similar results were obtained after LHEP immunization: mice that had received 10^8 BCG were far more resistant to local challenge with all doses of Lm than the mice immunized with lower doses of BCG. In fact, mice immunized with 10^6 or 10^5 BCG were resistant only to the lowest dose of Lm. It is concluded that the detection of macrophage activation by means of Lm is critically dependent upon the challenge inoculum. In general, the lower the challenge inoculum, the more sensitive the assay. In this study the 10^3 Lm inoculum was optimum.

S4-3

[illegible] PERITONEAL MACROPHAGES OF BEIGE MICE WITH CHEDIAK-HIGASHI [illegible]

[illegible] NAKAN[illegible], [illegible] SAITO-TAKI, K. ONOZUKA, Department of Microbiology, [illegible] Tochigi-ken 32[illegible], Japan.

[illegible] mice with Chediak-Higashi Syndrome were much more susceptible [illegible] virulent Salmonella enteritidis No.11 strain than parental C57BL [illegible] mice, and they had less bactericidal capacity towards the organism. [illegible] peritoneal exudate cells (PEC) obtained from the mice were cultured in the [illegible] absence of immuno-stimulants, such as N[illegible] dibutyryl guanosine 3':5' [illegible] monophosphate (dBcGMP), bacterial lipopolysaccharide (LPS) [illegible] N[illegible] acetylmuramyl-L-alanyl-D-isoglutaminyl-N[illegible] stearoyl-L-lysine (MDP-Lys(L18)) for [illegible], and then these cells were infected with No.11 organism for [illegible] min. After [illegible] the cells were cultured for 3 hr and the bactericidal capacity of the PEC [illegible] assessed by quantitative cultivations of viable bacteria in the phagocytes. [illegible] The previous treatment with dB[illegible]MP corrected the bactericidal capacity [illegible] PEC obtained from beige, but not from control mice. The treatment of PEC [illegible] MDP-Lys(L18) enhanced the bactericidal capacity of PEC obtained from [illegible] and control mice. Simultaneous treatment with dBcGMP and LPS or MDP [illegible] greater effects than any treatment by either alone. The [illegible] dBcGMP and N[illegible] dibutyryl adenosine 3':5' cyclic monophosphate (dBcAMP) [illegible] the effect of dBcGMP, while the augmentation of bactericidal [illegible] MDP-Lys(L18) was not affected by dBcAMP. These results [illegible] that the effects of LPS and MDP-Lys(L18) were not related directly to the [illegible] cyclic nucleotide regulation in beige macrophages.

S4-4

[illegible]

[illegible] Institute of Infectious Diseases, Laboratory [illegible] Netherlands.

[illegible] the peritoneal cavity of Listeria-treated mice [illegible] of the bacteria in the macrophage [illegible]

[illegible] were harvested after intraperitoneal [illegible] peroxidatic activity [illegible]

[illegible] the peritoneal cavity of Listeria-treated mice, the [illegible] macrophages were distinguished by the [illegible] peroxidatic activity and the appearance of phagocytosed [illegible] macrophages, the number of damaged bacteria was higher than that of [illegible] bacteria. [illegible] between peroxidase positive [illegible] and bacteria [illegible] was infrequently seen if bacteria were intact, [illegible] damaged. On the contrary, in mature macrophages, resident macrophages [illegible] most bacteria were intact.

These findings document significant differences among mouse peritoneal macrophage [illegible] with regard to listericidal activity.

S4-5

[illegible] BILIRUBIN AND BILE ACIDS ON OXYGEN-DEPENDENT BACTERICIDAL ACTIVITY OF [illegible] PHILS. M. IWANAGA, A. NAKAGAWARA, F. IKEDA. Department of Pediatric [illegible], Faculty of Medicine, Kyushu University, Fukuoka 812, Japan.

[illegible] tibility to infection is one of the most serious prognostic factors [illegible] patients with hyperbilirubinemia. Our previous study has revealed that [illegible] (NBT) of patient with biliary atresia had an impaired intracellular [illegible] killing activity, which accompanied with the decrease in generation [illegible] oxide anion (O_2^-) without change in myeloperoxidase (MPO) activity. In [illegible], we studied the effects of bilirubin and bile acids on O_2^- generation, [illegible] MPO activity of human NFs.

[illegible] generation induced by phorbol myristate acetate was measured by superoxide [illegible] dependent cytochrome c reduction at 550-540 nm at 37°C, and the cytolysis [illegible] trypan blue exclusion. MPO was measured by o-dianisidine method.

[illegible] incubation of human NFs for 4 hrs with 20 μM of unconjugated bilirubin increased [illegible] acetate (PMA) induced cytolysis and inhibited O_2^- generation to 0.02% ± 0.02% [illegible] cells (mean ± SD, n=7) (2.7% of control). On the other hand, bile acids [illegible] chenodeoxycholic acid, taurocholic acid, glycochenodeoxycholic acid and tauro- [illegible] acid had almost no effect on the O_2^- generation though the latter two [illegible] the effect depending on the concentration. MPO activity was not affected [illegible] and bile acids.

[illegible] suggested that bilirubin but not bile acids might be the main [illegible] oxygen-dependent bactericidal activity of NFs in hyperbilirubi- [illegible]

S4-6

[illegible] SUPPRESSION AND ANTAGONISM FOR OXYGEN INTERMEDIATES OF CYTOTOXICITY [illegible] H. SANIKAWA, M. MIYATA, Y. AOYAMA, S. OHGA, [illegible] Fukushima Medical College Fukushima 960.

[illegible] of PMNs was assayed on 51Cr cells naturally (NK) and on the antibody [illegible] cells (ADCC) at 37°C. Oxygen intermediates of O_2^- and H_2O_2 were also [illegible] PMNs. Cytotoxic activity of PMNs was assayed by 51Cr release most, [illegible] of superoxide dismutase (SOD), catalase, prostaglandin (PGE_1 [illegible] were co-incubated with PMNs and change of cytotoxicity of PMNs was evaluated. To [illegible] prostaglandin, O_2^- and H_2O_2 were also measured. SOD at concentration of [illegible] μg/ml did suppress the NK activity of both PMN and lymphocytes up to 50% of [illegible] untreated cells. Catalase at concentration of 120 μg/ml suppressed [illegible] the NK activity of PMNs but not of lymphocytes. Both PGE_1 and PGE_2 could [illegible] NK activity of both PMN and lymphocytes up to 80% of the original values [illegible] concentrations of 0.002 or 0.02 μg/ml. PGE_1 and PGE_2 could also suppress the [illegible] activity at concentration of 0.02 μg/ml up to 60% of the original value in PMN [illegible] up to 40 to 50% of the original value in lymphocytes. ADCC activity of PMNs [illegible] obtained from joint fluids of patients suffering from rheumatoid arthritis was similarly suppressed by both PGE_1 and PGE_2 at concentration of around 0.2 μg/ml. [illegible] reduction of PMNs stimulated by the opsonized zymosan was suppressed up to 50% by PGE_1 and to 50% by PGE_2 at concentration of 0.002 μg/ml. H_2O_2 production of PMNs was similarly suppressed by both PGE_1 and PGE_2 up to 60% at concentration of 0.002 μg/ml. It would be postulated that prostaglandins could suppress cytotoxicity of PMNs through reduced production of O_2^- and H_2O_2.

S4-7

N-FORMYL-METHIONYL-LEUCYL-PHENYLALANINE-INDUCED SUPEROXIDE RELEASE OF CALCIUM-DEPLETED HUMAN NEUTROPHILS. M.NAKAGAWARA, K.TAKESHIGE, H.UMIMOTO, S.SAITAKA, T.MINAKAMI. Departments of Anesthesiology and Biochemistry, Kyushu University School of Medicine, Higashi-Ku, Fukuoka, 812, Japan

The superoxide-release and the change in the intracellular free calcium ions on stimulation with N-formyl-methionyl-leucyl-phenylalanine were studied in human neutrophils deprived of divalent cations by treatment of the cells with an ionophore A23187 in the presence of ethyleneglycol-bis-(β-aminoethylether N,N'-tetraacetic acid. The depleted cells showed no release of superoxide on stimulation with the chemotactic peptide when calcium ions were absent in the medium but the activity was completely recovered when the cells were preincubated with calcium for at least 3 min before the stimulation. The recovery with calcium ions was dependent on the time of the addition relative to the time of the stimulation with the peptide. A simultaneous addition of both calcium and the peptide elicited about half of the full activity, while no release was observed when calcium was added later than 2 min after the stimulation with the peptide, though a marked elevation of intracellular free calcium monitored by quin-2 fluorescence was found. Comparison of the time-courses of the superoxide-release and the change in quin-2 fluorescence suggest that besides the elevation of the intracellular free calcium, a transient reaction which is also dependent on calcium is required for the full induction of the superoxide-producing activity.

S4-8

ENHANCEMENT OF OXYGEN CONSUMPTION OF NEUTROPHILS BY VANADATE.
Y. OZAKI, S. KUME, I. OHASHI. First Dpt. of Int. Med., Faculty of Medicine, University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo

The effect of vanadate, known to inhibit Ca-ATPase, was evaluated on oxygen consumption and oxygen radical production of human neutrophils. Neutrophils from healthy donors were collected by dextran sedimentation and Ficoll-Conray gradient centrifugation. Oxygen consumption was measured with a Clark type oxygen electrode from Yellow Springs Instrument, Inc. Superoxide production (O_2^-) was measured by the cytochrome c method, and hydrogen peroxide (H_2O_2) was measured, using the homovanillic acid fluorometeric assay. Oxygen consumption of neutrophils induced by fMLP, a chemotactic peptide, and by PMA, a tumor promotor, was increased by 200 % and 25 %, respectively, in the presence of 1 mM vanadate, whereas A23187-induced oxygen consumption was not enhanced by vanadate. O_2^- production by those stimulators were inhibited by vanadate in a dose-dependent manner. H_2O_2 production by fMLP was unchanged by vanadate, but A23187-induced H_2O_2 production was inhibited by vanadate. These observations suggest that the metabolic changes caused by these stimulators are different from one another and that vanadate may stimulate the production of certain oxygen radicals other than O_2^- and H_2O_2.

P1-1

[illegible] OF ACID MACROPHAGE ENZYMES IN EXPERIMENTAL PULMONARY [illegible]. MANGLA-BHAN, M.L. MEHROTRA AND P.K. VERMA. Allahbadia [illegible] Institute, University of Delhi, Delhi 110007, India.

Microbicidal capacities of activated alveolar macrophages were compared with [illegible] intracellular lysosomal hydrolases in pulmonary tuberculosis to determine [illegible] of these enzymes in antituberculous defence. Six enzymes expected to act [illegible] with M. tuberculosis were studied; N-acetyl glucosaminidase, β-galac- [illegible] and lysozyme were estimated by histochemical methods, and arabinosidase, [illegible] and phosphatase were estimated by a fluorescent staining method [illegible] this laboratory. Normal and BCG vaccinated guinea pigs were infected [illegible] with M. tuberculosis. Alveolar cells were harvested at intervals [illegible] and 4 weeks and stained histochemically. Number of bacilli [illegible] cellular enzyme content were determined for 600 cells. Phago- [illegible] cells were divided into two categories: those containing < 5 and those [illegible] bacilli. Extent of enzyme content of these cells was categorized [illegible]. It was observed that cells containing > than 5 bacilli/cell [illegible] cell, than in [illegible] cells for all six enzymes, in both [illegible] vaccinated animals, indicating an inverse relationship of number of [illegible] cell enzyme content. The study indicates that lysosomal hydrola- [illegible] phosphatases may be one of the factors involved in immunity in [illegible].

P1-2

IMMUNOLOGICAL CONSEQUENCES OF HOST-PARASITE MEMBRANE INTERACTIONS IN HUMAN FALCIPARUM MALARIA. C.F. Ockenhouse, M.J.Stewart, S.Schulman, W.L.Shear. NY Medical Center, NY,NY 10016.

The membrane interaction of human mononuclear phagocytes and erythrocytes infected with the malaria parasite, Plasmodium falciparum, was studied. Cytoadherence of parasitized erythrocytes to monocytes was observed, and the interaction occured via red cell membrane protrusions called knobs. This antibody-independent cytoadherence was specific since neither uninfected erythrocytes nor a knobless clone of parasitized erythrocytes bound to the monocytes. Trypsinization of K+ parasites abolished binding. Cytoadherence of K+ parasitized erythrocytes triggered a respiratory burst in monocytes and γ-interferon activated human macrophages as revealed by chemiluminescence, nitroblue tetrazolium reduction, and the electron microscopic cytochemical localization of reactive oxygen speices at the junction of juxtaposed membranes of parasitized erythrocytes and effector cells. Electron microscopy revealed that the consequences of this interaction resulted in degenerating intraerythrocytic parasites with the concurrent loss of knob structure. Evidence for oxygen-independent parasiticidal factors in the inhibition of parasite multiplication was obtained by co-culturing oxidatively deficient γ-interferon activated macrophages with the parasites. We postulate that the interaction of parasite-derived erythrocytic membrane determinants with host effector cells results in the release of cytotoxic molecules and may partially account for immunity to malaria.

PI-3

[illegible] OF THE LATEX PARTICLES BY THE ENDOTHELIAL AND KUPFFER CELLS IN THE [illegible] RAT LIVER. [illegible]DAN, K.WAKI. Department of Anatomy, Faculty of Medicine, [illegible] Medical and Dental University, Yushima, Bunkyo-ku, Tokyo, 113 Japan.

The sinusoidal endothelial and Kupffer cells of the liver constitute a part of the reticuloendothelial system, having ability to take up various substances. In the endothelial cells, however, the size of ingested latex particles is limited to [illegible] μm in vivo and 0.6 μm in vitro (D.F.Praaning-van Dalen et al 1982). In our observation, the latex particles of 0.11, 0.46 and 0.80 μm in diameters were taken up by the endothelial cells, when the liver was perfused with oxygenated [illegible] bicarbonate. Uptakes of the particles were observed at the luminal cell surface of the periphery or at the thick portions of the endothelial cells. After 5 min perfusion the nascent phagosomes were covered with large patches of the bristle coat. After 1 hr the phagosomes fused with lysosomes. The cells especially distributed in the peripheral zone of the hepatic lobules showed active endocytosis of the latex. The number of the ingested particles in the endothelial cells, however, was much less than that in the Kupffer cells. In vivo experiments, [illegible] uptakes of latex particles was observed in the endothelial cells. While in the Kupffer cells, particles were incorporated by the ruffle membranes or sank into the cytoplasm without the large patches of the bristle coat in the perfusion system as well as in vivo. We conclude that the sinusoidal endothelial cells are [illegible] to ingest of the 0.7 μm latex particle in the perfused liver, but the [illegible] of uptake are quite different from that of Kupffer cells.

PI-4

FOAMY MACROPHAGES ASSOCIATED WITH ERYTHROPHAGOCYTOSIS. T.ISHIHARA, F.UCHINO, T.YAMASHITA, [illegible]OKA, M.[illegible]ABASHI, S.MATSUMOTO. First Department of Pathology, Yamaguchi University School of Medicine, and the School of Allied Health Sciences, Yamaguchi University, Ube, 755, Japan.

It is well known that foamy macrophages (foamy cells) frequently appear in the reticuloendothelial system in various kinds of pathologic conditions. In this study, foamy cells associated with accelerated erythrophagocytosis were experimentally induced in mice by subcutaneous injection of murine red cell membranes or glutaraldehyde-treated red cells, and time course observations were made by light and electron microscopy with special reference to the mechanism for the formation of foamy cells. Following injection of red cell membranes, increasing numbers of foamy cells were induced in the subcutaneous tissue, and most of them contained myelinlike materials in their cytoplasm. The glutaraldehyde-treated red cells were also phagocytosed by the macrophages, in which engulfed red cells were subsequently fragmented into small spherules with increased density. As intracellular digestion progressed, these spherules showed loss of hemoglobin content that was replaced by fine granular flocculent material. At this stage, such macrophages revealed foamy appearance in light microscopy. We conclude that the increased red cell destruction in the reticuloendothelial system is one of pathologic states in which foamy cells are formed.

P1-5

P1-6

[illegible] cells in the lymph node sinuses are identified under the electron [illegible] their processes surrounding reticular fibers with elastic fibers. [illegible] of large doses of horse radish peroxidase and compound 48 80 into the [illegible] the rat induced mast cells to degranulate in the popliteal lymph node. Reticular cells took up actively many released mast cell granules which showed acid metachromasia by toluidine blue stain on the epon-embedded semithin sections. Macrophages in the sinus also phagocytized released mast cell granules, but larger number of granules were found in reticular cells than in macrophages. In order to follow the fate of the taken-up granules, the acid phosphatase activity was detected in macrophages and reticular cells by the method of the Gomori under the electron microscope. The acid phosphatase activity was demonstrated at the taken-up mast cell granules in macrophages, though no activity of this enzyme was detected in those of reticular cells. This means that phagolysosomes are formed and mast cell granules are digested in macrophages but not in reticular cells. The complex of heparin and protamin was also taken up by both reticular cells and macrophages, and the activities of acid phosphatase were demonstrated at the site of the complex in both types of cells. Therefore it is suggested that reticular cells in lymph node sinuses take up and digest foreign substances in lysosomes as macrophages, but do not digest their own mast cell granules.

P1-7

Phagosome-lysosome fusion in human macrophages' first encounter with M. leprae.
C.M. Scollard, I.D. Gardner. Chiang Mai/Illinois Leprosy Research Project, Chiang Mai, Thailand, and University of Hong Kong, Hong Kong.

We have examined the initial interaction between monocyte-derived macrophages and M. leprae in vitro, to determine whether phagosome-lysosome fusion (PLF) is stimulated or is inhibited, as occurs with some other intracellular pathogens. M. leprae were obtained directly from skin biopsies of active, untreated lepromatous leprosy patients, stored at 4°C and used within 30 days. Peripheral blood monocytes from healthy, non-leprosy-exposed volunteers were obtained by adherence to glass and cultured in medium with 20% autologous plasma. The cells were labelled with ferritin on the 3rd day in vitro, and M. leprae were inoculated on Day 4. Cells were examined ultra-structurally 3 and 5 days after inoculation to determine PLF. Preliminary results show ferritin in 156 of 172 phagosomes, indicating phagosome-lysosome fusion in 91% of instances following phagocytosis of M. leprae. The bacilli observed in these phagolysosomes usually showed evidence of damage, possibly as a result of lysosomal enzyme activity. Since PLF does not appear to be inhibited by M. leprae, the intracellular survival and growth of these organisms appears to be due to resistance of their vital functions to lysosomal enzymes and other toxic effects within the lysosomes.

P1-8

[redacted]

PI-9

ASSAY METHOD FOR ACTIVE PHAGOCYTOSIS OF POLYMORPHONUCLEAR LEUKOCYTES BY FLUORESCEIN LIBERATION FROM PHAGOCYTOZED BEADS

[illegible]

[illegible]

PI-10

[illegible] MECHANISM OF DESTRUCTION IN VIVO [illegible]

[illegible], [illegible] and K. Miyaji, Second Division of Internal Medicine, [illegible] Hospital, Sakai, Nara, Japan.

Autologous erythrocytes (RBCs) from Rh-positive subjects were labeled with ^{51}Cr-99m and coated with IgG by treating them with anti-D serum (D-RBC) and then returned to the subjects with ^{51}Cr-labeled NEM- (N-ethylmaleimide) treated RBCs for simultaneous measurement of their respective kinetics in vivo.

Despite the D-RBCs' discocytic form and normal resistance to gradient density centrifugation, alteration of the intrasplenic kinetics of D-RBCs manifested at first in increase in the proportion of them to demonstrate slow dynamics, which related proportionately to elevation of their extraction ratio in the spleen.

Light and scanning electron microscopic pictures demonstrated entrapment in the cord of D-RBCs which had been infused intraarterially just after removal and well perfusion washing of the spleens from cases of ITP or portal hypertension. The picture of D-RBCs phagocytosed by cordal macrophages was observed on TEM in the spleen, into which D-RBCs had been infused two hours prior to removal. The picture of D-RBC in *transit* through the sinus wall in bilobed form was more abundant than that of NEM-RBC, which demonstrated reduced osmolar resistance and deformability.

Effect of high dose intravenous gamma globulin, supposingly a blocker of the macrophage Fc-receptor, was examined in 9 ITP cases, in whom prolongation of platelet survival in postmedication stage was associated with coincidental reduction of extraction ratio of D-RBCs. The TEM picure of the spleen removed in such stage demonstrated a feature of predominant myelin-like residuals in degradation process of phagocytosed platelets in macrophages suggesting recent suppression of accelerated phagocytosis.

PI-11

[illegible] ON METABOLISM IN THE RETICULAR CELLS AND MACROPHAGES OF THE RAT LYMPH NODE SINUS: A STUDY BY ELECTRON MICROSCOPY. K. TAKAYA, K. MAYAMA and T. TAKAGI.
Toyama Medical and Pharmaceutical University, 2630 Sugitani, Toyama 930 01

The reticular cells of the lymph node sinus can be distinguished from neighbouring macrophages by their processes enclosing reticular fibers containing elastic fibrils with the junctional complex at the tips of plasma membranes. Alcohol administration induced accumulation of iron containing large dense granules in the cytoplasm of ferritin in the cytosol of the reticular cells. After injection of native ferritin in the rat footpad, they were accumulated preferentially in the lysosomes of the cytoplasm of the reticular cells. They contained iron and lead after acid phosphatase reaction, which was confirmed by EDS and XRMA X-ray microanalysis. Dextran iron injected in the footpad were accumulated selectively in the macrophages of the popliteal lymph node sinus. Intraperitoneal injection of an iron chelator, desferrioxamine induced depletion of cortical follicles and paracortex of the lymph node, post capillary venules approaching the subcapsular sinus and accumulation of macrophages and reticular cells in the sinuses. Fragments of cell debris, probably of the dead lymphocytes were revealed only in the macrophage cytoplasm of the lymph node sinus of the rats treated with deferoxamine for two month. A large number of toluidine blue metachromatic granules were found in the cells of the spleen of these rats. Injection of native ferritin in the footpad of the rats made the granules appear in the reticular cell and macrophages of the lymph node sinus. The two types of cells in the lymph node sinus operate in the defense of the animals through iron metabolism in different mechanisms, which was revealed by electron microscopy.

PI-12

[illegible]

PI-13

DIRECT MEASUREMENT OF INTRACELLULAR REACTIVE OXYGEN BY MICROSPHERE-BOUND LUMINOL. T. [illegible], T. [illegible], S. [illegible]. Basic Research Laboratories, Toray Industries, Inc.*, Toray Research Center**, 1111 Tebiro, Kamakura 248, Japan.

Highly reactive oxygen released from Freund's complete adjuvant-elicited murine peritoneal macrophages into phagosomes was measured by luminol dependent chemiluminescence (CL). Reactive oxygen within phagosomes was able to be measured directly by a new method, utilizing microsphere-bound luminol. It was confirmed by the following results that microsphere-bound luminol CL is generated by phagosomal reactive oxygen; 1) when macrophages had been treated with cytochalasin B but ethanol, the stimulation of the macrophages by microsphere-bound luminol produced very little CL, despite the increase in the amount of extracellular reactive oxygen. 2) CL production remained slight even though the cytochalasin B-treated macrophages were stimulated by both of phorbol-12-myristate 13-acetate (PMA) and microsphere-bound luminol. By use of microsphere-bound luminol, the effect of lipopolysaccharides (LPS) on macrophages was studied. When the incubation of macrophages with microsphere-bound luminol was preceded by the overnight culture with LPS (1ng/ml-100μg/ml), the CL intensity was reduced, depending on the LPS concentration. This result suggests that the phagosomal reactive oxygen related microbicidal activity is reduced by LPS.

PI-14

OXYGEN INTERMEDIATES IN THE PATHOGENESIS OF SHOCK. S.M. REICHARD, N.M. BAILEY, Medical College of Georgia, Augusta, GA 30912.

An analysis of reduced glutathione (GSH) in RES tissue supports the hypothesis that toxic oxygen products from activated phagocytes are associated with impaired bactericidal activity and survival in shock. Following tissue injury GSH levels in the splenic, pulmonary and intestinal tissue were lowered, decreasing the mechanism by which oxygen free radicals are detoxified. Damaged intracellular structures may obstruct the delivery of myeloperoxidase to the phagolysosome, accounting for loss of bactericidal activity and the escape of toxic oxygen products from the cell may cause tissue damage. NADPH oxidase was also found to be lowered affecting production of H_2O_2 further reducing the bactericidal activity. To test the hypothesis, a variety of agents that interfere with or scavenge oxygen radicals were administered in vivo. GSH (200 mg/kg i.p.) replacement immediately following trauma, prevented these adverse sequelae. Methylprednisolne (30 mg/kg i.v.) which inhibits the production of O_2^- and H_2O_2, given 2 hr before injury increased survival, as did dimethylsulfoxide (4.5 g/kg i.p.) a specific OH^0 scavenger, given 30 min before trauma. Desferrioxamine (200 mg/kg i.p.) an iron chelator which inhibits the conversion of H_2O_2 to OH^0 in the presence of iron, given 30 min prior to injury also enhanced survival data. It is concluded that toxic oxygen intermediates not only kill bacteria, but when released from phagocytic cells damage the surrounding tissue and affect mechanisms concerned with the pathophysiology of shock.

Tuesday, September 4

S5-1

[illegible]

S5-2

TITLE [illegible] EPITHELIOID CELL GRANULOMAS
[illegible] CELL FORMATION AND IMMUNOLOGICAL COMPETENCE

AUTHORS [illegible]
Hosp. For Trop. Dis. [illegible] and Tokushima University, Japan*

Experimental Mycobacterial granulomas due to BCG in rat skin indicates that tubercle formation arises from an interplay of antigen (load), antigen - antibody ratio, cell mediated immunity and delayed hypersensitivity. The most rapid resolution of antigen with resolution is effected by early recruitment of activated mφs and BCG [illegible] minimal. The next best performance is in tubercles due to BCG [illegible] reformed complexes in antibody excess, when a constant rate of monocyte influx and transformation to activated macrophages is maintained until resolution. The slowest rate of healing is in primary lesions and is associated with the whole range of mature monocytes to epithelioid cells. Loose clusters of cells with infiltrating lymphocytes and good vascularity are features of the rapidly resolving lesion as opposed to tight compartmentalisation and diminished vascularity in the slow resolving tubercle.

The results of light microscopy, ultrastructure and bacteriological analysis of this study are presented.

These observations are discussed in their relevance to leprosy, leishmaniasis and tuberculosis where similar situations of immunological competent and incompetent epithelioid cell tubercles are seen due to M. leprae, Leishmania species and M. tuberculosis.

S5-3

EXPERIMENTAL PULMONARY FOREIGN BODY GRANULOMATOUS INFLAMMATION AND ANERGY. [illegible] Allred, K. Kobayashi and T. Yoshida. Dept. of Pathology, U. of Conn. Health Center, Farmington, CT 06032

A murine model of pulmonary foreign body granulomatous inflammation (FBGI) was developed to aid in the study of the mechanism of this type of inflammation and to explore the consequences of these lesions on other inflammatory/immune host responses. Female balb-C mice were injected intratracheally with neutral cross-linked dextran beads (Sephadex G50) and sacrificed at intervals. Large epithelioid granulomas developed around the beads which were quantitated by measuring the radius of inflammation on routine light microscopic sections. Conspicuous granulomas were present within 24 hours, peaked by 2-3 days and rapidly declined. An absence of immunogenicity of dextran in this form was demonstrated in several ways emphasizing the true foreign body nature of the granuloma. The general status of cell mediated immunity in granuloma bearing animals was assessed by measuring 24 hour footpad (FP) swelling induced by intradermal injection of lymphocyte mitogens. Marked suppression of PHA and Con-A elicited FP responses was associated with early FBGI. FP reactivity recovered by 2 weeks following bead injection. Aqueous extracts prepared from FBGI lungs could passively transfer suppression of the mitogen FP response when injected intraperitoneally into normal mice. In conclusion: (1) Dextran beads induce large noninfectious pulmonary granulomas in mice. (2) A state of transient anergy exists in animals bearing active dextran granulomas. (3) This anergy appears to be the result of a soluble mediator(s) produced in the inflamed lungs. Supported by NIH grants [illegible] and HL-29382.

S5-4

THE ROLE OF INTERLEUKINS IN GRANULOMATOUS INFLAMMATION AND THE ASSOCIATED ANERGY. [illegible], K. KOBAYASHI, C.ALLRED, AND R.CASTRIOTTA. Department of Pathology, University of Connecticut Health Center,Farmington, CT 06032, USA

The mechanisms of development of granulomatous inflammation and the associated anergy remain unknown. To explore the role of lymphokines and interleukins during the formation of pulmonary granulomas, BALB/c mice were immunized with methylated bovine serum albumin (MBSA) in complete Freund's adjuvant, and challenged intratracheally with MBSA-coated agarose beads to induce pulmonary granulomas. Granulomas started to appear within one day after the injection and reached its peak on day 3, when aqueous extracts from these lungs were found to contain IL 1 and MIF in the absence of IL 2. Both the suppression of cutaneous DTH response and the diminution in the antigen-stimulated lymphocyte proliferation in vitro occurred concomitantly with the development of lung granuloma. The production of IL 2 by antigen-stimulated lymph node cells was also found depressed in these animals. These results suggest that macrophages probably activated by lymphokines in vivo produce IL 1 in the granulomatous lesion and that the observed cutaneous anergy seems to be mediated by circulating lymphokines, which may be responsible for the suppressed production of IL 2 by lymph node lymphocytes. Supported by NIH grants HL-29382 and HL-01171.

SS-5

[illegible] T. BABA, [illegible] Institute of Clinical Medicine and [illegible] Nursing, University of Tsukuba, [illegible]

[illegible] normal human monocytes was studied. [illegible] were incubated with the culture media [illegible] normal human sera. [illegible] aggregation and formation of [illegible] with those incubated with normal human sera. [illegible] revealed that the molecular [illegible] aggregating factor were [illegible] and [illegible] the monocytes and [illegible] aggregating factor was responsible for formation [illegible] with the [illegible] factor, [illegible] spreading activity [illegible] supernatants. [illegible] result, [illegible] demonstrated.

[illegible] contribute to the formation [illegible] of monocytes or formation [illegible]

SS-6

[illegible] MACROPHAGE [illegible] IN INTERSTITIAL [illegible] M. [illegible] Institute of Clinical Medicine, University of Tsukuba, [illegible]

[illegible] in healthy subjects and the patients with interstitial lung [illegible] (AM) [illegible] BAL fluid were [illegible] AM were cultured for 48 [illegible] FN [illegible] localization of FN was examined [illegible] labeled antibody to [illegible] in the BAL fluid and the culture supernatants were measured by ELISA. FN was demonstrated in [illegible] perinuclear space, Golgi apparatus and [illegible] on the cell surface of AM. [illegible] patients had slightly higher FN [illegible] in the BAL fluid than healthy subjects. Moreover, FN levels in the culture supernatants [illegible] were [illegible] in healthy subjects [illegible] hypersensitivity pneumonitis [illegible] interstitial pneumonia [illegible] in pulmonary fibrosis with collagen diseases [illegible]. These results suggest that: (1) FN is produced by AM and [illegible] localized in their secretory organelle, (2) AM-derived FN may be one constituent [illegible] FN, (3) the production of FN by AM is significantly increased in [illegible] patients with interstitial lung diseases compared with in healthy subjects. We [illegible] that human AM-derived FN might participate in the disease process of the [illegible] and granulomas observed in these pulmonary disorders.

S5-7

TINY SILICATE CRYSTALS FOUND IN MACROPHAGES OF PLEURAL FLUID OF ASBESTOS-EXPOSED PATIENTS. S. Y. KIMURA(1), H. MIURA(2) (1)Tsukuba University, Ibaraki-305, JAPAN, (2) Yokosuka Kyosai Hospital, Kanagawa-238, JAPAN

To clarify the morphogenesis of the pleural plaque and/or mesothelioma of the parietal pleura in asbestos-exposed patients, we examined various cells and tissues of 60 patients who had been exposed to asbestos, using light and polarized microscope. We also used energy-dispersive x-ray microanalyser to analyse element composition of deposited crystals and of natural asbestos stones as crocidolite, chrysotile, and amosite. The ferruginous bodies showing drum-stick shape were found in the alveoli and peripheral lung parenchyme. The double refractile crystals (DRC) were detected in alveolar macrophages, interstitium, and lymph nodes in all patients. Furthermore, 7 patients had DRC in the parietal pleura and the macrophages in the pleural fluid. One patient had many crystals in the liver and spleen. An x-ray microanalysis revealed that DRC had silicon, aluminium, calcium, magnesium, and iron. Asbestos stones also contained DRC in their long fibers and each DRC showed specific element composition for each asbestos stone. These crystals both in lungs and stones were ranging in 2 to 10 micra length and in 0.5 to 1 micron width. We conclude that the pleural mesothelioma and/or plaque are resulted from some stimuli carried by the macrophages from the alveolis. The pleurisy of the man, who had asbestos-related occupation, is possibly due to silicate crystals as well as the tuberculosis. The relationship between these crystals and the initiation of mesothelioma still remains in a riddle.

S5-8

[redacted]

S6-1

[illegible] ...TION OF HUMAN ALVEOLAR MACROPHAGES IN LYMPHOCYTE PROLIFERA- [illegible] ...WATANABE, R.I. [illegible] NODA, S.HASEGAWA, M.FUJIMA. Institute [illegible] Medicine, [illegible] University of Tsukuba, Ibaraki, [illegible], Japan

[illegible] function of human macrophages (MΦ), we [illegible] alveolar macrophages (AM) and blood monocytes (M[illegible]) on proliferat[illegible] mitogens (PHA, con A) and antigen (P[illegible]). AM were [illegible] obtained by bronchoalveolar lavage. AM and M[illegible] were [illegible] lymphocytes (L) at various MΦ:L ratios. [illegible] measurement of H-thymidine uptake [illegible], AM and M[illegible] augmented proliferative response [illegible] mitogens. At high MΦ:L ratios [illegible], AM marked[illegible] optimal and suboptimal doses of mitogens, while M[illegible] at optimal dose of mitogens and enhan[illegible] mitogens. At lower MΦ:L ratio[illegible], AM were [illegible] proliferation at almost same level [illegible] MΦ:L ratios, AM and M[illegible] strongly suppressed the responses. [illegible] MΦ:L ratios was partially relieved [illegible] AM stimulated with lipopolysaccharide [illegible] without adding AM. These results [illegible] modulating effect on lymphocyte proliferation [illegible] depend on the presence of AM [illegible] effect of AM [illegible] effect of AM are partially mediated [illegible]

S6-2

[illegible] CELL LINES WITH APC ACTIVITY.

[illegible]

[illegible], Japan [illegible]

[illegible] have been established from [illegible]. They all adhered [illegible] of murine macrophages, with [illegible], lysozyme and latex bead phagocytosis, and the [illegible] sheep erythrocytes. It was found by [illegible] these lines bearing Ia molecules (RI-SL1, [illegible]). The percentage of I-A positive cells varied between [illegible] and [illegible] with [illegible] and I-E antigens on the cell surface were able to be induced [illegible] lymphokine containing medium. Since Ia molecules on APC are [illegible] presenting antigens [illegible] cell, these lines were tested for [illegible] for the activation of antigen-specific T cell prolifera- [illegible] and for the [illegible] of T helper cells in antibody production by B cells. [illegible] I-A positive clones were capable of stimulating proliferation of [illegible] antigen-specific T cells, and furthermore, the T cells activated by those [illegible] were [illegible] anti-TNP-PFC response. On the other hand, RI-SL4, I-A negative clone, failed to activate T cells.

[illegible] with both I-A positive and negative clones, would be [illegible] immunological responses.

56-3

ENHANCEMENT OF MONOCYTE ACCESSORY CELL FUNCTION BY INTERFERON β. S. BECKER. University of North Carolina, Chapel Hill, NC 27514.

Human monocytes respond to interferon β (IFN) by increasing their surface density of HLA-DR (Ia) 3-4 fold. The monocytes can be exposed to IFN at 0°C, which suggests that receptor occupation is sufficient to induce the signal required for increased Ia synthesis. Cytoplasmic HLA-DR specific mRNA is increased 4 fold in the monocytes within 8 hours after IFN exposure. If this is due to message stabilization or increased transcription is presently under investigation.

The implications of this increase in Ia on the accessory cell function of the monocytes has been investigated. Both autologous stimulation and presentation of soluble antigen is increased in IFN treated monocytes. The stimulatory index is proportional to the number of Ia molecules expressed. Increased accessory cell function is especially noticeable at low monocyte to T cell ratios. Time course experiments evaluating the induction of T cell proliferation show that ^{3}H thymidine incorporation can be detected at least one day earlier with the IFN treated monocytes. Stripping the monocyte surface of Ia antigen with monoclonal antibody inhibits proliferation. These observations suggest that the effect of IFN on monocytes is to enhance their accessory function most likely via enhancement of Ia expression.

56-4

S6-5

FUNCTIONAL PROPERTIES OF CULTURED MURINE THYMIC MACROPHAGES, RELEASE OF IL-1 AND INDUCTION OF MHC RESTRICTED PROLIFERATION OF (T-G)-A-L SPECIFIC T CELL LINE.
R. GALILY*, O. AXELROD**, E. MOZES**. *Immunology, Hebrew University, Jerusalem, **Chemical Immunology, The Weizmann Institute of Science, Rehovot, Israel.

We have recently shown that successful long-term culture of proliferating C57Bl/6 thymic macrophages can be achieved by plating adherent thymic cells in the presence of L-cell conditioned medium on dishes coated with an extracellular Matrix. The adherent cells proliferate for more than 60 days in vitro. We identified the cells as mononuclear phagocytes by the following criteria: phagocytosis of bacteria, positive staining for non-specific esterase and the presence of fc receptors and F4/80, a specific macrophage cell surface marker. A high percentage of these cultured cells bear Ia surface antigen (65-96%). Our present study shows that thymic macrophages secrete significant levels of PGE_2 constitutively. Further, LPS stimulation prompts high level secretion of interleukin-1 (IL-1). Thymic macrophages show tumoricidal activity following activation with either LPS alone or in combiration with T-cell lymphokine. Thymic macrophages are capable of antigen presentation in a MHC restricted fashion to a (T-G)-A-L specific T-cell line as assessed by T-cell proliferation. No proliferation was seen in the presence of unrelated antigen. The response could be inhibited by the appropriate monoclonal anti-Ia reagents. Our results indicate a close interrelationship between thymic macrophages and T cells, especially as regards macrophages presentation of antigen. The system which involves, homogeneous populations of thymic macrophages obtainable in large numbers, offers a unique opportunity to study the cellular and biochemical requirements for antigen processing and presentation.

S6-6

[illegible] Ia-POSITIVE ANTIGEN-PRESENTING CELLS IN TUMOR-BEARING HOSTS
[illegible] NAKAMURA, [illegible] Department of Public Health, Sch. Med., Kitakyushu, [illegible] Japan

[illegible] analyze the mechanism of immune suppression in tumor-bearing hosts, [illegible] antigen-presenting activity of spleen macrophages from C3H/He mice bearing SSK63 [illegible] was studied in vitro. The indicator T cells for the antigen-presenting macrophages were INP-specific proliferative T cells which were induced by the restimulation of lymphnode T cells from picryl chloride-painted mice with INP-coupled syngeneic macrophages in vitro and INP-specific killer T cells which were induced by culturing spleen T cells with INP-coupled syngeneic macrophages in vitro. Both T cell activities were markedly impaired when T cells from tumor-bearing mice were used. The antiven (INP) presenting activity of macrophages for both T cells from normal mice was also impaired when macrophages from tumor-bearing mice were used. Furthermore, the production of interleukin 1 by macrophages stimulated with LPS was impaired in tumor-bearing mice. The dysfunction of macrophages in tumor-bearing mice was not due to the development of suppressor cells, but due to the decrease of Ia-positive macrophages. Thus, it is suggested that one of the mechanism of immune suppression in tumor-bearing hosts is a dysfunction of Ia positive antigen-presenting cells.

S6-7

I-J POSITIVE LINED MACROPHAGES REPLACE THE SPLENIC ACCESSORY CELLS IN THE INDUCTION OF SUPPRESSOR T CELLS. R. M. NAKAMURA*, A. NAGAYAMA** AND T. TOKUNAGA* *DEPT OF TUBERCULOSIS, NIH, TOKYO, AND **DEPT OF MICROBIOL, SAGA MEDICAL SCHOOL, SAGA, JAPAN.

Lined macrophages SL-1 are I-A and I-J positive, while the other lined macrophages SL-4 are Ia negative. Both lined cells were from C3H/HeN and transformed with SV40. We have reported that I-J positive splenic adherent cells are necessary for the induction of suppressor T cells against delayed-type hypersensitivity (DTH) to BCG in vitro. To avoid the contamination of T cells to the macrophages, SL-1 cells were used instead of the splenic adherent cells of C3H in this system. The SL-1 cells were mixed with normal C3H T cells and 50 μg of PPD per ml and cultured for 4 days. The nonadherent cells were transferred into cyclophosphamide-treated C3H and the recipients were immunized to BCG immediately. DTH was determined 2 weeks later by the foot pad reaction to PPD. The mice receiving the cells from the culture of SL-1 and C3H T cells showed significantly suppressed DTH , while those receiving the cells from the culture of SL-4 and C3H T cells did not. When the SL-1 cells were treated with anti-I-J^k and complement, the suppression was eliminated. Treatment with anti-I-A^k did not affect the activity of SL-1 in the induction of suppressor T cells in vitro. Taken together, I-J positive lined macrophages played a role of the accessory cells in the induction of suppressor cells against DTH. These results confirmed the conclusion that I-J positive macrophages are necessary for the induction of suppressor T cells against DTH to BCG.

S6-8

[illegible] CELLS INCLUDING PLASTIC DISH ADHERENT CELLS IN MURINE BONE MARROW CHIMERA. M.IMAMURA, H.FUJIMOTO, M.KAWAI, M.OKABE, K. ARIKADA, I.MIYAKAWA. The Third Department of Internal Medicine, Hokkaido University School of Medicine, Sapporo 060, Japan.

Bone marrow cells from BALB/c mice, which were not treated with anti-Thy 1 anti-serum plus complement, were transplanted into lethally X-irradiated C3H/He mice, either intrasplenically (i.s.) or intravenously (i.v.). The prolonged survival time of C3H/He mice transplanted i.s. was observed when compared the survival time in two groups. To determine whether suppressor cells were generated in chimeric mice, co-cultured experiments were set up. Spleen cells from (BALB/c → C3H/He) i.s. chimeras showed suppressor activities against both BALB/c anti C3H/He MLR and BALB/c anti C57BL/6 MLR, although spleen cells from (BALB/c →C3H/He) i.v. chimeras also showed this kind of suppressor activities. According to characterization studies, there was no definite difference between suppressor cells in i.s. chimeras and that in i.v. chimeras so far. They were composed of T cells and non T cells, plastic dish adherent cells and non adherent cells, and radioresistant cells and radiosensitive cells. Thus, it was suggested that several kinds of suppressor cells were generated in the spleen of chimeras. It is of interest to determine which suppressor cells are most important to induce and maintain transplantation tolerance.

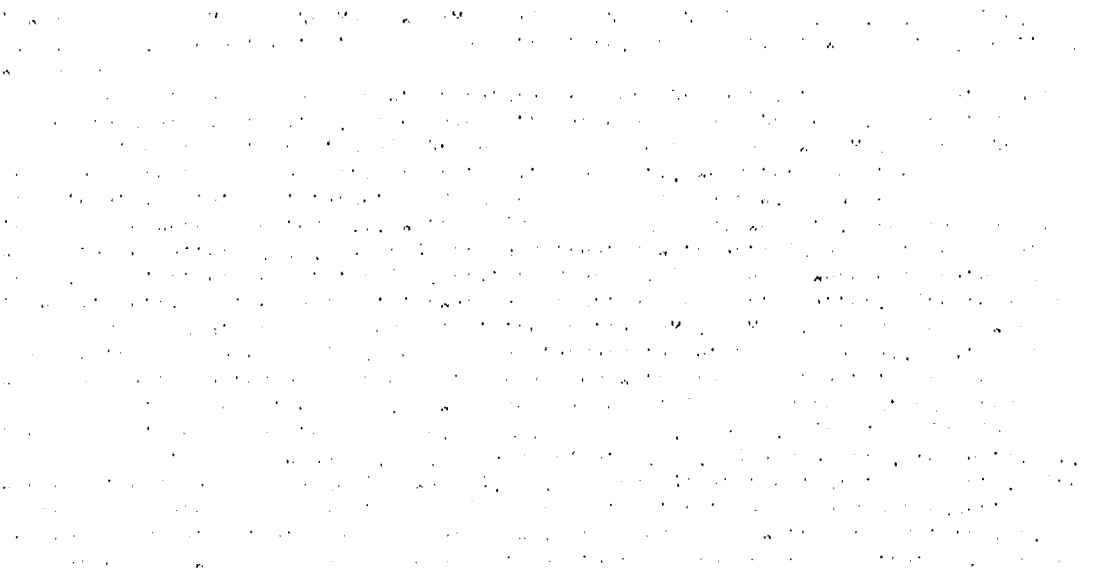
S7-1

S7-2

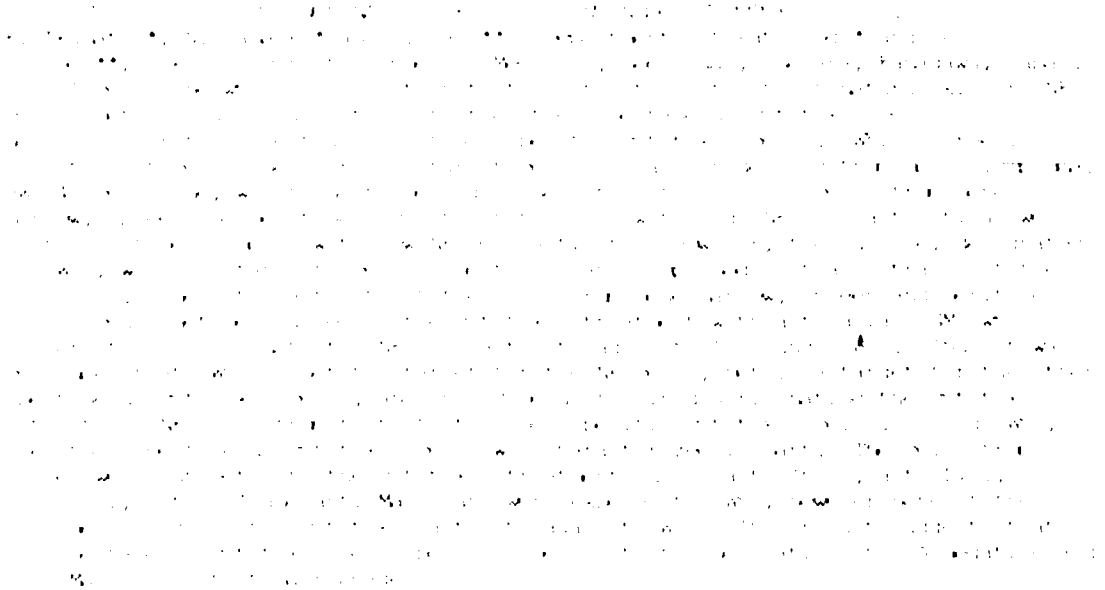
57-3

57-4

S7-5



S7-6



S7-7

[illegible] KILLING OF HUMAN BLOOD MONOCYTES: RELEASE OF MONOCYTE [illegible]TOXIC FACTORS (MCF) DURING INTERACTION WITH TARGET CELLS

[illegible] Paterson [illegible]
[illegible] Holt Radium Institute, Manchester M20 9BX, U.K.

[illegible] blood monocytes purified by adherence to plastic dishes [illegible] coated with [illegible] but not with fetal calf serum, from normal donors [illegible] a variety of tumor cells, including natural [illegible] NK [illegible] short term (4 h) ^{51}Cr release assay. On [illegible] the [illegible], NK cells are [illegible] release soluble cytotoxic factors (NKCF) during [illegible] NK [illegible] target cells. Similar cytotoxic factors were released from blood monocytes isolated by [illegible] serum coated plastic dishes. [illegible] produced by coculture of blood monocytes and K562 resulted in lysis of [illegible]. The activity of MCF was optimally detected [illegible] using a [illegible] uptake after 40 h incubation with K562 [illegible] activity was detected [illegible] containing MCF was obtained by short term coculture of [illegible] monocytes with NK sensitive target cells. Monocytes [illegible] with NK [illegible] target cells [illegible] to release such cytotoxic factors. MCF was specific to NK sensitive target cells (K562, Molt 4), with no reactivity to NK resistant target cells [illegible]. MCF activity was substantially removed by [illegible] on NK sensitive target cells. No differences were observed in specificity and kinetics of [illegible] and action of NKCF and MCF. These results suggest that MCF may be comparable to NKCF and involved in the lytic mechanism of blood monocyte mediated natural [illegible].

S8-1

S8-2

ONTOGENY OF MACROPHAGE COLONY-FORMING CELLS (M-CFC). T. J. MacVITTIE. Armed Forces Radiobiology Research Institute, Bethesda, MD 20814.

Analysis of the ontogeny of mononuclear phagocytes has been facilitated by the application of in vitro colony-forming assays for granulocyte-macrophage (GM) colony-forming cells (CFC). Detection of another CFC specific for production of macrophages (M-CFC) prompted us to investigate the ontogeny of this CFC in hemopoietic and lymphoid organs. M-CFC were assayed in cell suspensions prepared from bone marrow (BM), spleen (SPL), liver (Liv), peripheral blood (PB), and thymus (T) tissue at various times from fetal, neonatal, and adult, female B6D2F1 mice. M-CFC and GM-CFC were detected using the double layer agar technique with pregnant mouse uterine extract as the source of CSA. Progeny of M-CFC were examined morphologically through specific stains, electron microscopy, presen of fc receptors, and phagocytosis. M-CFC were detected in all organs and PB assayed. Fetal and neonatal M-CFC exhibited the same general characteristics of adult tissue-derived M-CFC. Fetal Tissue: 12-day livers contained large numbers of M-CFCs while SPL, BM, and PB had detectable levels at 14 15 d. M-CFC in these organs increased slowly through fetal growth. M-CFC content was always greater than that of GM-CFCs in all organs assayed. Thymic M-CFC were detected as early as 14 d. TM-CFC content increased 9-fold while no GM-CFC were detected. Neonatal Tissue: M-CFC content of all organs and PB incresed significantly through the 14 d following birth, with the exception of liver, which showed a marked rise through 48 hours after birth, decreasing to nondetectable levels by d 14. Adult Tissue: Stable levels of M-CFC were detected after 6 weeks of age. M-CFC content was significantly greater than GM-CFC in all hemopoietic organs and PB and was present in liver, thymus, lymph nodes, serous cavities, alveolar space and brain tissue.

S8-3

[illegible]

S8-4

[illegible]

S8-5

[illegible]

S8-6

ULTRASTRUCTURAL ANALYSIS OF RELATIONSHIP BETWEEN LEU 7^{+} CELLS AND DENDRITIC RETICULUM-CELLS IN GERMINAL CENTERS OF HUMAN LYMPH NODES. E. YUDA, M. DOBASHI, Y. IMAI

[illegible] lymph nodes were fixed in periodic iodine-lysine-paraformaldehyde fixative. For ultrastructural study, natural killer cells and T cell subsets were immunohistochemically detected in an indirect method by incubating the 50μ thick sections with Leu 7, OKT3, OKT4, OKT8 and other hybridomas antibodies, followed by combinding peroxidase-labeled anti-mouse Ig. For light microscopical analysis, in histochemical double staining of other surface antigen coexistence on Leu 7^{+} cells, 5μ thick cryostat sections were used. The sections were incubated with Leu 7 (IgM) and other surface markers (IgG) [illegible] and by combinding alkaline phosphatase-labeled anti-mouse IgM and peroxidase-labeled anti-mouse IgG respectively.

Results: Leu 7^{+} cells distributed predominantly in germinal centers (GC), especially in the light zone and border portion between the corona and GC. In GC, OKT3^{+} cells, OKT4^{+} cells and OKT8^{+} cells were also located in these regions. Ultrastructurally Leu 7^{+} cells in GC were of medium-sized to large lymphoid cells poor in intracytoplasmic granules. Their cytoplasmic extensions focally interwoven demonstrated a close spatial relation with antigen-antibody trapping labyrinth structures of dendritic reticulum cells. In double staining Leu 7^{+} cells in GC showed co-expression with OKT3 and OKT4 antigens. No reactivity of them with OKT8 denoted a difference from Leu 7^{+} cells outside GC. These findings showed that they were differ, phenotypically and morphologically from Leu 7^{+} cells in peripheral blood causing highly natural killer activity previously reported. They might be OKT3^{+}, OKT4^{+} -helper inducer T cells with Leu 7 antigens, and participate in immunological regulation of GC cell-proliferation.

S8-7

LANGERHANS TYPE DENDRITIC CELLS IN THE LYMPHNODES OF NUDE MICE. H.UDA,S.TANAKA, *.MARUYAMA*. Dept. of Pathology,Kagawa Medical School.,*Shionogi Laboratories.

Working hypothesis that epidermal Langerhans cells(Lc) originate from bone marrow,migrate into the epidermis,function antigen-trapping,leave through dermal lymphatics,reach regional lymphnodes and present antigen to T-lymphocytes has not been controversial. We reported absolute increase in the regional lymphnodes of the skin of BALB/C,nu/nu mice electron-microscopically and morphometrically(J.Leukocyte Biology 1984).This phenomenon may be accumulation rather than proliferation,because epidermal Lc in the skin of nude mice are normal morphologically and quantitatively. Lc of nude mice were found most frequently in the marginal sinuses and subsequently in the paracortical region with some distance from the postcapillary venules.Some Lc found in the latter were stained darkly and sustained degenerative changes.Lc in the lymphnodes have indistinguishable appearence from interdigitating cells(IDC) of the paracortex exept the existence of Langerhans cell granule(LcG) and however different from the ordinary macrophages. Lc have markedly indented nucleus,pale cell plasma,rich parallel filaments,numerous vesicle and not a few but small phagosome.Not infrequently cored tubule(Kobayashi & Hoshino) which are thinner and bend or circular granule coexist with LcG in the Lc of nude mice.They were found frequently in the lymphnodes of mice suffered from contict dermatitis. Lc-rich suspension were gained by light pipetting from the medium incubated for 12-24 hours in the cultured dish of lymphnode cells suspension of nude mice(approximately 20 cells). They are weakly adhesive cells and have a remarkable resemblance to IDC or monocytes light-microscopically.

S8-8

IMMUNOHISTOCHEMICAL STUDY OF DENDRITIC RETICULUM CELL IN LYMPH FOLLICLE OF THYROID. M. YAMAKAWA, T. KASAJIMA, Y. IMAI
Yamagata Univ. School of Medicine, Yamagata, Japan 990-23

It knows that the germinal center(GC) of lymph follicle are seen often in tissue section of the various thyroid lesions, especially autoimmune thyroiditis. Immunohistochemically the GC in thyroid was studied to elucidate immunological behavior.

The 68 human specimens presenting GC were studied and compared with that of the lymph node and tonsil. In this study, following antibodies were used: rabbit anti-human IgM F(ab')2 fragment, IgG F(ab')2 fragment, IgA, S-100 protein, thyroid-associated; thyroglobulin(Tg), thyroxine, thyroxine-binding-globulin, thyroid-stimulating hormone, complement(F(ab')2 fragment ; C1q, C3c, C3d, C3activator, C5, C9, properdin, and monoclonal mouse anti-human dendritic reticulum cell(DRC , C3bR, HLR2, Leu and OKT series etc.

Various degree of positive staining between IgM,IgG,C1q,,C3d,DRC and C3bR were observed in lacy pattern within the GC of thyroid, similar to that in the lymph node or tonsil. Electron microscopically, Tg, IgM, IgG, C1q and C3d binded the cell surface and cytoplasmic labyrinth structure of DRC in GC of thyroid.

It concludes that the GC of thyroid approximately resemble to that in the lymph node or tonsil, as far in structure and function. Apart from the question whether Fc receptor on the DRC participate, it appears that on the cell surface and cytoplasmic labyrinth structure, DRC carrys out trapping, retaining and degradation of immune complex, mediating some complement receptors, and plays a important role in immune response.

PII-1

DEVELOPMENT OF SPLENIC ELLIPSOID AND ITS CELLULAR CONSTITUTION IN CHICK EMBRYO. T. Asai, T. Sassa, T. Koshikawa, F. Furuta*. Laboratory of Germfree Life Research Institute for Disease Mechanism & Control, Nagoya Univ. School of Medicine, Nagoya 466 and *Poultry Disease Laboratory, National Institute of Animal Health, Gifu 501-11, Japan

Specific pathogenfree chick embryos(PDI-1) were employed for the ontogenic studies on the splenic ellipsoid. On 7th day of the incubation at 38°C primitive vascular structure appeared among the mesenchymal frame work of the spleen. The sheath artery which was characterized by its high endothelial cells could be detectable at the embryonal age of 10 days, while the ellipsoid still undeveloped. The development of the ellipsoid was approaching completion on 17th day of the incubation. The endothelial cells of the sheath artery connected together tightly with a junctional complex until this period. Therefore, any carbon particles injected via a blood vessel in the yolk sac did not leak out from the sheath artery before the completion of sheath development. There were observed many granulocyte around the sheath artery about day 11 of embryonic development. However, they decreased in number as the ellipsoid developed. Certain number of macrophages which demonstrated sufficient activities of acid phosphatase and phagocytosis could be found in the spleen at early embryonal period(11 days of the incubation),when the bone marrow did not appear. The ellipsoid consisted mainly of the reticulum cells beside the sheath artery and of macrophages in its marginal zone. Any lymphocytes were not observed before the 19th day of chicken eggs.

PII-2

[illegible] MONOCYTE SUBSETS RELEASE DIFFERENTIALLY DIFFERENT HUMAN PERIPHERAL BLOOD MONOCYTE. [illegible] M.J.H. MOOLENBEEK, J. FLORIJN, P.J.M. BOUMA, J. HAMPE, J. LEEMANS, W.S. HART, [illegible] Netherlands Cancer Institute, Plesmanlaan [illegible], Amsterdam, The Netherlands.

We [illegible] monocyte which differ both [illegible] activity. In the [illegible] we demonstrated the [illegible] metabolites after stimulation [illegible] (CL). It was found that [illegible] were [illegible] active than those with the [illegible] M monocytes were cultured for periods of 2, 4 and 18 [illegible] was affected [illegible] hours before CL [illegible] 13 % and 4 %, respectively. To establish [illegible] monocyte fractions were related to [illegible] we measured the amount of the [illegible] and TBX, produced after 24 hours of incubation. No [illegible] TBX, which is [illegible] component produced by monocytes, [illegible] These results indicate that [illegible] functionally different subpopulations. Furthermore [illegible] to produce both in a positive and negative [illegible] monocyte subsets cannot be explained by [illegible]

P II-3

P II-4

GRANULOCYTE-MACROPHAGE PROGENITOR CELLS IN THE LIVER OF HUMAN EMBRYOS.
Y. OHNISHI AND M. KITAZAWA, 2nd Dept. of Path., Niigata University School of Med. Asahimachi-dori, Niigata 951, Japan.

The density of granulocyte-macrophage progenitor cells (CFU-GM) in the liver of 34 human embryos and 10 fetuses was examined by in vitro colony assay. The cell suspension to culture was produced from the whole hepatic cells. CSFs were human placental conditioned medium (HPCM) and human leucocyte phytohemagglutinin stimulated conditioned medium (PHA-LCM). The culture was performed by the modified method by Pike & Robinson. After one week, the whole cultured agar-gel was fixed, and stained on slide glass by May-Giemsa, naphthol ASD chloroacetate esterase, alpha naphthyl butyrate esterase, and those double esterase stain. Colonies were classified as granulocyte colony (G colony), macrophage colony (M colony), and granulocyte macrophage mixed colony (GM_c colony).

The total average of CFU-GM / $2x10^5$ whole hepatic cells was counted 21.5 by HPCM and 21.1 by PHA-LCM. The number of CFU-GM showed high titer in 7 weeks of menstrual age, but diminution in 8 weeks and then almost constance in low titer. On closer examination, CFU-GM appeared in small number at 0 day in 7 weeks of menstrual age (corresponding to 35 days of embryonal age), increased at 2 days (crown-rump length 9.5mm, corresponding to 37 days) and showed maximum titer at 5 days (corresponding to 40 days). The rate of the M colony predominated in the earlier stage, but it decreased during the advantage of embryonal age and G colony turned to predominant in the later stage in the system using HPCM. Using PHA-LCM, proportion of the M colony predominated in every stages, but its of G colony showed a tendency to increase gradually.

PII-5

Ultrastructural feature of the lysozyme-containing cells of the rat. H. ARIMA, N. MORI, M. KOJIMA. Basic Medical Sciences, University of Tsukuba, Tennodai 1-1-1, Sakura, Niihari, Ibaraki 305, Japan.

Rabbit antihuman urinary lysozyme has been shown to cross react with rat tissues. The present study was to characterize the ultrastructural feature of the lysozyme-containing cells among monocyte-macrophage series of normal adult and fetal tissues, and of subcutaneous BCG granuloma of the rat. For localization of lysozyme, a direct electron-microscopical labeled antibody technique was applied. Rabbit antiserum to human urinary lysozyme was obtained commercially from the Behringwerke A.G. Rat tissues without antiserum were used as controls.

In the normal rat, lysozyme was localized in the primary granules of promonocytes, and in Pc, nuclear envelope(NE), rER and golgi cisternae of promonocytes. It was also demonstrated in Ne, rER, GC and vesicles(V) in a small number of subcutaneous histiocytes and of lymph node macrophages(Mφ), and in almost all of alveolar Mφ and in a large number of exudate peritoneal Mφ. Lysozyme was not clearly demonstrated in the fibroblasts, Kupffer cells and resident peritoneal Mφ. In the rat fetus, lysozyme was detected in Ne and rER of subepidermal cystic cells from 15 days of gestation, and of fetal Mφ in the subepidermal tissues after 17 days of gestation. Cells did not react to antilysozyme serum. In the BCG granuloma, lysozyme was localized in blood exudate monocytes, in Pc, NE and rER of exudate Mφ, and in Ne, rER, GC and V of mφ, epithelioid cells and Langhans' giant cells. In the control, no cells of monocyte stained positively.

These findings suggested that lysozyme-containing cells among monocyte-macrophage series of the rat belonged to cells of mononuclear phagocyte system.

PII-6

HYALOCYTE: A PECULIAR CELL THAT BELONGS TO MONONUCLEAR PHAGOCYTE SYSTEM. Y. TAKADA, T. ISHA, T. TAKEUCHI, K. MIZUNO and H. WATELLA. Department of Ophthalmology, Hokkaido University School of Medicine, Sapporo 060, JAPAN.

There exists a non-vascular cell population in primary vitreous of the eye, called as hyalocyte. It has been reported that hyalocytes have phagocytic functions and have lysosomal enzymes in the cytoplasm. It, however, still remains obscure whether these cells originate from blood monocytes, that is, belong to the cells of mononuclear phagocyte system. In the study herein we examined guinea pig hyalocytes by electronmicroscopy, cytohistochemical and immunohistochemical methods. Electronmicroscopically hyalocytes had a irregular nucleous with moderate condensed chromatin. In cytoplasma primary and secondary lysosomes were seen. Histochemical staining showed that hyalocytes are positive in nonspecific esterase, ATPase, PAC and acid phosphatase, but weak or negative in peroxidase in light microscopy. Immunohistochemical study revealed that hyalocytes are negative in surface immunoglobulin, but some cells are Ia antigens of MHC class II antigens positive.

These results are consistent with the concept that hyalocyte belongs to the cells of mononuclear phagocyte system.

P11-7

DEVELOPMENT AND MATURATION OF FETAL RAT MACROPHAGES IN ONTOGENESIS. K. TAKAHASHI, M. NAITO, F. YAMAMURA, N. SUEYOSHI. Second Department of Pathology, Kumamoto University Medical School, Kumamoto 860.

Fetal rat macrophages were fine structurally characterized by abundant polyribosomes, variable-sized vacuoles, lysosomes, a small number of rough endoplasmic reticula and long filopodia. The macrophages bore Fc receptor and complement (C3) receptor on their cell surface, were capable of immune phagocytosis and possessed ability to adhere to foreign body surfaces. These cells began to appear in the liver anlage, subepidermal mesenchyme, brain and other tissues from approximately 13 days of gestation, had a high mitotic activity, particularly in the early fetal period, and were gradually matured with the lapse of gestation, showing increased numbers of lysosomal components, decrease in amount of polyribosomes and transformation into an ameboid cell. In hepatic hematopoiesis, such macrophages proliferated vigorously, showed endogenous peroxidase activity in rough endoplasmic reticula and nuclear envelope from about 16 days of gestation and were matured and transformed into Kupffer cells when gestation ended. In the subepidermal mesenchyme, fetal macrophages proliferated notably in the early fetal period and were also matured and transformed into histiocytes. Such maturation processes of the fetal macrophages obviously differ from those of monocytic cell lineage. In peripheral blood, similar macrophages were found. Thus, hepatic hematopoiesis is regarded as a major source of supplying macrophages to various tissues prior to the initiation of bone marrow hematopoiesis. Furthermore, development of fetal macrophages was demonstrated in blood islands of volk sac hematopoiesis, and similar macrophages were observed in blood capillaries of the subepidermal mesenchyme prior to the beginning of hepatic hematopoiesis.

Thursday, September 6

S9-1

S9-2

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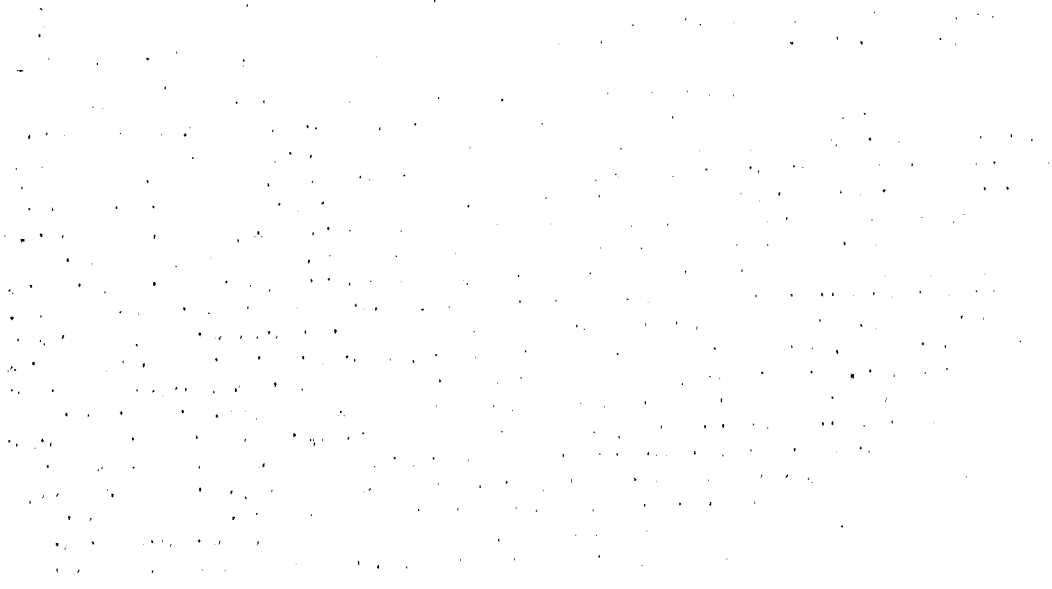
59-6



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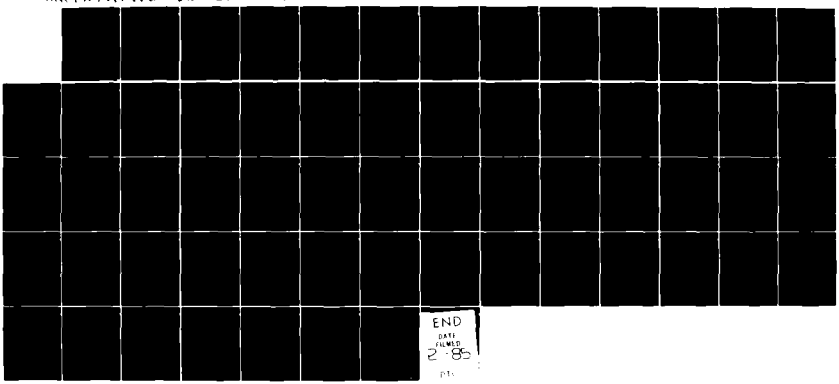

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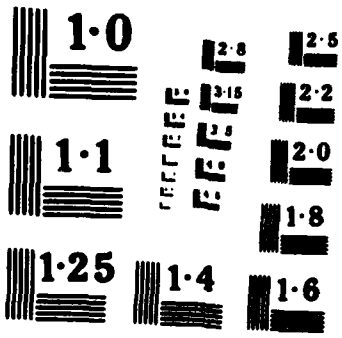
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S9-7

ANTIGENIC AND AMINO ACID SEQUENCE HOMOLOGY BETWEEN HTLV AND THE RETROVIRUS ENVELOPE PROTEIN p15E. G. Cianciolo, T. Palker, R. Kipnis, B. Haynes, and R. Snyderman. Howard Hughes Med. Inst., Duke Univ. Med. Ctr., Durham, N.C. 27710, USA.

The transmembrane retrovirus envelope protein p15E has been shown by both serological analysis and amino acid sequencing to be well conserved in retroviral evolution. We have previously shown that murine retroviral p15E inhibits the accumulation of murine macrophages to inflammatory foci in vivo and the responses of human monocytes to chemotactic stimuli in vitro. Others have found that feline p15E inhibits tumor immunity in cats and the in vitro blastogenic responses of both feline and human lymphocytes, perhaps by blocking interleukin-2 production. We therefore sought to determine if the immunosuppressive human T-cell leukemia-lymphoma retrovirus, $HTLV_I$, shares antigenic homology with p15E. A highly specific rabbit antiserum to p15E was prepared using affinity-purified Rauscher leukemia virus p15E antigen. With this antiserum, we examined detergent-disrupted $HTLV_I$ and envelope-enriched preparations of $HTLV_I$ by 1) immunoprecipitation of ^{125}I-labeled viral proteins by antibody and Staph. A. followed by SDS-PAGE and 2) SDS-PAGE of viral proteins followed by western blotting and incubation with antibody and ^{125}I-protein A. Rabbit anti-p15E recognized both 46 Kd and 61 Kd proteins thought to be associated with the $HTLV_I$ envelope. Furthermore, comparison of the published amino acid sequences of the $HTLV_I$ envelope and both murine and feline p15E by the PROTHOM computer program revealed a sequence of 26 amino acids which contains a significant amount of homology (73%). These data suggest that a p15E-like component of the HTLV envelope could, in part, be responsible for the immunosuppression accompanying diseases associated with infections by the family of HTLV viruses.

S10-1

MACROPHAGES AND TUMOUR BIOLOGY D.S. NELSON Kolling Institute, RNSH, St Leonards, NSW, 2065, Australia

Activated macrophages, capable of recognizing and selectively destroying tumour cells, are probably delivered to sites of tumour cell deposition in vivo by reactions similar to those of delayed-type hypersensitivity (DTH). Macrophages can also destroy tumour cells by antibody-dependent cell-mediated cytotoxicity. In the absence of antibody, normal macrophages can, however, potentiate tumour growth. With co-cultures of mouse tumours and mouse peritoneal macrophages this was shown by measuring tritiated thymidine incorporation, ^{125}IUdR incorporation and cell numbers and by flow cytometry. Stimulation of tumour cell proliferation required cell contact and was inhibited by trasylol and dexamethasone. The susceptibility of cultured tumour cells to stimulation varied cyclically.

On the other hand, tumours may evade immunological attack by producing soluble factors that inhibit DTH. Immunization of mice with phenol-saline extracts of tumours was found to confer resistance to the depression of DTH and partial resistance to the growth of challenge tumours. The factors responsible appear to share some determinants with a retrovirus structural protein.

S10-2

THE ORIGIN OF GAUCHER CELLS AND ULTRASTRUCTURAL COMPOSITION OF THEIR STORED MATERIAL. M. NAITO, K. TAKAHASHI, H. HOJO, H. JINNOUCHI. 2nd Department of Pathology, Kumamoto University Medical School, Kumamoto, and 1st Department of Pathology, Fukushima Medical College, Fukushima, Japan.

Gaucher cells are considered to be a cytologically transformed macrophage with intralysosomal accumulation of tubular structures, because they were proved to bear Fc and complement (C3) receptors on the cell surface and to be capable of immune phago cytosis. High resolution electron microscopy in negatively stained preparations and freeze fracture replicas revealed that the tubular structures consisted of gently twisted or straight multilayers. Glucocerebroside biochemically extracted and purified from surgically removed spleens from patients with Gaucher disease showed similar layered appearances. These findings suggest that the tubular structures are composed of glucocerebroside molecules and are formed by accumulating the molecules in the form of flat layers.

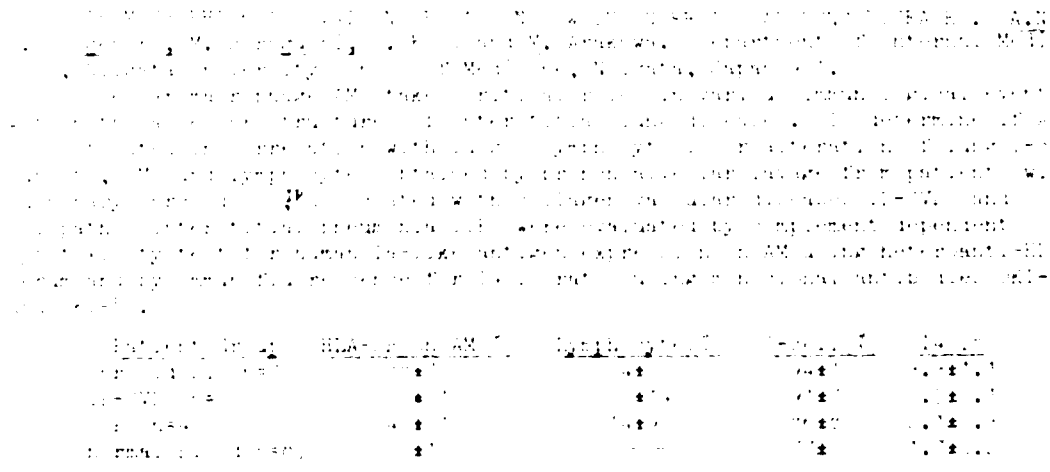
For the purpose of clarifying the origin of Gaucher cells, blood monocytes from a Gaucher patient and control subjects were cultured and examined electron microscopi cally. The monocytes from the patient and controls transformed gradually into macro phages when cultured in the medium containing 10% horse serum and in the medium saturated with glucocerebroside. Within a couple of days after phagocytosis of heat denaturated human erythrocytes, a small amount of tubular structures are found to be developed in phagolysosomes of Gaucher monocytes, but no tubular structures ap peared in any control monocytes. After ingestion of tubular structures purified from the spleen of Gaucher patients, both the Gaucher and control monocytes transformed into Gaucher cells.

S10-3

CHARACTERIZATION OF FOAM CELLS AND PARTICIPATION OF MACROPHAGES IN ATHEROGENESIS. F. TOMITA, K. TAKAHASHI, M. SAITO, S. FUKUDA. Second Department of Pathology, Kumamoto University Medical School, Kumamoto 860.

In order to elucidate the cytological characters and origin of foam cells in atherogenesis, the aortic lesions of cholesterol-fed rabbits and Watanabe hereditary hyperlipemic rabbits (WHHR) were investigated ultrastructurally and immunocytochemically. Among the foam cells in the lesions, two major cell populations were distinguished. One was proved by rosetting assays to bear Fc receptor and/or complement (C3) receptor on the cell surface and to be capable of immune phagocytosis, whereas the other was positively stained with peroxidase-antiperoxidase method for desmin, the intermediate filament type specific for muscle cells. The former is considered to be foamy macrophages, and the latter is presumed to be derived from smooth muscle cells. In the early stage of atherogenesis, blood monocytes were observed to enter the aortic lesions and foamy macrophages were found frequently in the intima, while foam cell transformation of smooth muscle cells predominated in the advanced stage and the transformed cells disclosed characteristics of macrophages to a certain extent. In addition, non-rosetted and desmin-negative foam cells were present, though a minor and probably heterogeneous population. As for lipid storage of foamy macrophages, lipid vacuoles with or without limiting membrane, myelin-like bodies, cholesterol crystals and ceroid-like granules were distinguished, and ingestion, lysosomal digestion and processing of LDL and accumulation of the lipids in the foam cells were demonstrated by the electron immunocytochemical method, using a peroxidase-labeled LDL antibody. Removal and digestion of the lipids in atheromatous lesions are thus thought to be the pricipal role of macrophages during atherogenesis.

S10-4



S10-5

Superoxide production of monocyte derived macrophage from collagen diseases.

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To evaluate the role of monocyte and macrophage system in the pathogenesis of collagen diseases, superoxide production of blood monocyte derived macrophage from collagen diseases were studied. Blood monocytes fixed on plastic dish were cultured in the serum free media RPMI 8089 for 3 days. On day 3, superoxide production of monocyte cultured in Eagle's MEM containing cytochrome C with or without PMA for 2 hours were measured. Superoxide production of monocyte derived macrophage from SLE (n 8) was 2.5 times (without PMA, 2 hours of incubation) and 1.6 times (with PMA, 2 hours of incubation) more than control. Other collagen diseases such as RA, polymyositis, PN, Behcet's disease showed also increased superoxide production of monocyte derived macrophage. These data suggest that the monocyte derived macrophage from collagen diseases are activated in vivo to produce and secrete more superoxide than control. Comparative studies of these data and other laboratory data will be discussed.

S10-6

DYSFUNCTION OF HLA-DR POSITIVE MONOCYTES IN SLE PATIENTS. F. SHIRAKAWA, T. YAMA-UCHI*, H. SUZUKI. Dept. 1st Internal Medicine and *Dept. Immunology, Nat. Inst. of Environ. Health, Kitakyushu, 807, Japan

Monocyte function of SLE patients was studied as accessory cells for the activation of T cells in vitro. Nylon column-purified T cells alone were not able to respond to PHA to proliferate and to develop suppressor cells, but the addition of T cells after of monocytes restored both T cell activity with dose dependent manner. This accessory function of monocytes was markedly impaired in SLE patients. The dysfunction of monocytes was marked in an active stage of SLE, but not in an inactive stage. The dysfunction of monocytes in SLE patients was not due to the appearance of suppressor cells, but due to the decrease of HLA-DR positive cells. Furthermore, antibodies specific for monocytes, but not for B cells, T cells and HLA-DR was detected in SLE patients, and which affected the function of monocytes. Thus, it is suggested that the dysfunction of monocytes plays an important role for the pathogenesis and the process of SLE.

S10-7

IMPAIRED ADHERENT CELL FUNCTION IN SODIUM PERIODATE ($NaIO_4$) ACTIVATION OF MONONUCLEAR CELLS (MN) FROM PATIENTS WITH SYSTEMIC LUPUS ERYTHEMATOSUS (SLE). R. LOWITZER, K. PHILLIPS, A.R. RABSON. Immunology Department, South African Institute for Medical Research, School of Pathology, University of the Witwatersrand.

Treatment of normal human mononuclear cells (MN) with $NaIO_4$ (0°, 30 minutes) results in lymphocyte blastogenesis which is assessed by measuring ^{3}H-thymidine incorporation by the activated cells. $NaIO_4$ induced MN cell activation involves an obligatory macrophage-lymphocyte interaction. When the reactivity of MN cells from SLE patients to $NaIO_4$ was investigated it has been found to be grossly impaired. In order to establish the cellular nature of this impairment we performed experiments in which adherent cells from normal donors were mixed with non-adherent cells from SLE patients and vice versa. Reconstitution of patients' lymphocytes with normal adherent cells resulted in a normal response to $NaIO_4$ while adding patients' adherent cells to normal lymphocytes caused a great reduction in the response to $NaIO_4$. These results suggest that the impaired reaction of SLE MN cells to $NaIO_4$ is due to an adherent cell dysfunction. In order to further define this dysfunction we added PMA (phorbol myristate acetate) or IL-2 containing supernatants, to patients' MN cells. In both cases correction of $NaIO_4$ response to normal levels occurred. Addition of IL-1 supernatants, however, only partially restored the $NaIO_4$ reaction. Our results taken together suggest that a defect in the accessory function of adherent cells and a related or separate defect in IL-2 production are responsible for the impaired reaction of SLE MN cells to $NaIO_4$.

S10-8

[illegible] FIBRONECTIN BY MONOCYTES AND ALVEOLAR MACROPHAGES IN PATIENTS WITH [illegible] SYSTEMIC SCLEROSIS. T. KON, T. KAWASHIMA, H. WATANABE, M. OTSUKA, F. AMANO, T. SAKURAI, H. KAWAKI, R. TSUNODA, M. KOJIMA. Institute of [illegible] Basic Medicine, University of Tsukuba, Ibaraki, 305, Japan.

Progressive systemic sclerosis (PSS) is a multisystem disease of unknown etiology characterized by fibrotic changes of skin and internal organs such as lung and gastrointestinal tract. Recently fibronectin, a high molecular weight protein, is reported to be produced by monocytes and macrophages and to play some role in fibrotic process. We studied production of fibronectin by monocytes and alveolar macrophages in patients with PSS. Peripheral blood monocytes were prepared from heparinized blood. Alveolar macrophages were obtained by bronchoalveolar lavage. Monocytes and alveolar macrophages were cultured and fibronectin in the culture supernatants was assessed by enzyme linked immunosorbent assay using peroxidase-labelled IgG antibody to human plasma fibronectin. Monocytes were found to produce fibronectin only after 4 days in culture. Amounts of fibronectin produced by monocytes during 7 days' culture were greater in PSS than those in normal controls. Alveolar macrophages from PSS patients demonstrated greater than normal production after 48 hours' culture. These results indicate that 1) monocytes may acquire secretory ability of fibronectin during maturation into tissue macrophages, 2) monocytes and macrophages are capable of producing significant amounts of fibronectin in PSS, 3) fibronectin thus secreted may have relevance to the fibrotic process in PSS by promoting macrophages adhesion and recruiting fibroblasts as a chemoattractant for these cells.

S10-9

The effects of immuno adjuvants on plasma fibronectin

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Fibronectin is a high molecular weight glycoprotein. It occurs in an insoluble form called as cellular fibronectin and a soluble form called as plasma fibronectin. Plasma fibronectin (PFN), as an opsonic protein, modulates reticuloendothelial phagocytic function. It is suggested that change of PFN is related to reticuloendothelial system and various immune system. The present study was undertaken to examine the change of PFN by administration of immune adjuvants to mice intraperitoneally. Purified fibronectin was obtained from pooled mouse plasma by affinity chromatography on a gelatin-Sepharose 4B. Antiserum of mouse FN was prepared by immunization of rabbits. PFN concentration was estimated by Laurell's electroimmuno assay. PFN increased in aged mouse but no difference between strain and sex was observed. PFN value was augumented by LPS, MDP, Lentinan and SPG. Phagocytic function of peritoneal exudate cells induced by Lentinan and SPG was increased than resident cells. Our data suggest that increased PFN value was associated to activation of macrophage.

S11-1

PRODUCTION OF THE LYMPHOCYTE STIMULATING FACTOR BY POLYMORPHONUCLEAR LEUKOCYTES.
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A lymphocyte stimulating factor was found in cell-free exudate fluid in an early stage (3-9 hrs) of a casein-induced peritoneal inflammatory site. The major cell population of these early peritoneal exudate cells was polymorphonuclear leukocytes (PMN). The early PMN were highly purified on a density gradient by Percoll. The purified PMN (99-99.99) were found to have a lymphocyte stimulating factor in their cytoplasm and released it on the appropriate stimulations in vitro, such as kaolin, staphylococci, aluminum hydroxide and chemotactic peptide, but not on stimulation with polystylene beads, formalinized sheep erythrocytes, muramyl dipeptide or lipopolysaccharide of E. coli (LPS). The blood PMN did not have the factor in their cytoplasm, but could be triggered to have it by stimulations such as shaking incubation, calcium ionophore or LPS. This induction process of the active factor production by blood PMN was dependent on the incubation time, temperature, and protein synthesis by the PMN. The active factor produced in the blood PMN could be released into the culture medium by the same stimulations as used for the inflammatory exudate PMN. The active factor in the PMN cytoplasm was similar in its physicochemical natures to the released PMN factor. This active factor was biologically similar to interleukin 1 because of its ability to induce the production of interleukin 2 (IL 2) for a subclone of EL-4 cells without any aid of lectin stimulation. It also induced the IL 2 production for peanut-agglutinin-receptor negative thymocytes, or Lyt 1 T cells when they were stimulated with lectin or alloantigen.

S11-2

PROPERTIES OF IgA IN POLYMORPHONUCLEAR LEUKOCYTES. Z.MOLDOVEANU, F.KOMIYAMA, I.MORO, J.MESTECKY. University of Alabama in Birmingham, Birmingham, AL 35294.

Polymorphonuclear (PMN) leukocytes express surface receptors for the Fc of IgA. With the use of immunofluorescence, immunoelectron microscopy, gel chromatography, electrophoresis and various radioisotope techniques, we determined levels and characterized the molecular properties of intracellular IgA in PMN from normal individuals and patients with alcoholic cirrhosis or IgA myeloma. Cell lysates of PMN from cirrhotic and myeloma patients contained higher levels of IgA than normal subjects, in accordance with higher serum levels of IgA in these patients. IgA in cell lysates of PMN from cirrhotic patients occured predominantly in a monomeric form, while that of normal subjects was mostly polymeric, as demonstrated by electrophoretic mobility of IgA in SDS gels, presence of J chain and the ability to bind secretory component. In contrast to plasma cells, IgA of both subclasses was detected in PMN. On incubation with PMN, polymeric IgA1, IgA2 or secretory IgA proteins were internalized more efficiently than monomeric IgA. When PMN from normal individuals were incubated with sera or PEG precipitable immune complexes from cirrhotics, IgA was found within vesicles of the PMN. The intracellular uptake of IgA was not species specific, because human PMN internalized human as well as mouse IgA. PMN have the ability to internalize IgA and IgA-containing immune complexes, and may be involved in the catabolism of IgA, particularly when the normal pathway of removal of IgA is impaired. (Supported by AI 10854).

S11-3

ALTERATIONS IN GRANULOCYTE (G) FUNCTION WITH CITRATE SOLUBLE (CS) AND INSOLUBLE (CI) NEPHROPATHIC IMMUNE COMPLEXES (IC). J. RILEY, G. BOCK, T. PHILLIPS, C. SMITH, S. BAEHR. Children's Hospital National Medical Center and George Washington University School of Medicine, Washington, DC 20010.

Pooled rabbit precipitating antiovalbumin-ovalbumin I-C were fractionated by solubility in citrate buffer (pH 4.0, ionic st. 0.26) and were studied in vivo by their glomerular deposition after IV injection in rats and in vitro by their effects on G aggregation (Agg), adherence to glass (Ad) and generation of chemiluminescence (chemi). The CS I-C localized in the capillary wall and paramesangial area while the CI I-C localized in the central mesangium. In the absence of serum, addition of CS I-C to G stimulated G-Agg and chemi without affecting G-Ad. With the addition of serum, CS I-C produced a further 60% increase in G-Agg ($p<0.001$) and chemi ($p<0.95$) while inhibiting G-Ad by 92% ($p<0.001$). In contrast, CI I-C in the absence of serum had no effect on these parameters. With the addition of serum, G-Agg was inhibited, G-Ad reduced by 31%, and chemi unchanged. The difference in inhibition of G-Ad by CI and CS I-C in serum was significant at $p<0.001$. In spite of the different in vivo localization and in vitro effect on G function, the CI and CS I-C were immunochemically identical by ultrasedimentation, complement fixation, isoelectric focusing and component analysis. We conclude from these data that I-C of differing citrate solubilities have different pathophysiologic effects both in vivo and in vitro. Studies of differential kinetics of Fc receptor binding, IgG subclass, complement component interaction and platelet activation are ongoing to investigate these differences.

S11-4

PHAGOCYTOSIS STIMULATORY SUBSTANCES RELEASED FROM PLATELETS. H. SAKAMOTO. Department of Pathology, Wakayama Medical College, Wakayama City, 640, Japan.

The effect of platelet release products (PRPr) on neutrophilic phagocytic activity was investigated. Release reaction from washed human platelets was induced by a high speed centrifugation in a glass tube. Human neutrophils were separated from heparinized blood by a discontinuous density gradient method in which cautions were taken against platelet contamination and release. Phagocytic activity of neutrophils attached on a bottom of microplate well was assessed after treatment with PRPr or other test materials. IgG sensitized sheep erythrocytes (IgG-EA) and complement coated IgM-sensitized sheep erythrocytes (IgM-EAC) were used for particles to be ingested.

Phagocytosis of both IgG-EA and IgM-EAC by neutrophils increased 2 to 3 times of control values after treatment with PRPr. Ultrafiltration analysis of PRPr revealed existences of two different groups of IgG-EA phagocytosis stimulators. One was a macromolecular substance larger than 10^5 daltons, the release of which was not inhibited by indomethacin. The other was low molecular weight lipid smaller than 500 daltons. Direct exposure of neutrophils with TxB_2, $PGF_{2\alpha}$, and PGE_2 enhanced the neutrophilic phagocytic activity of IgG-EA.

Phagocytosis of IgM-EAC was elevated by the low molecular weight substance in PRPr which was inactivated by apyrase. Direct exposure of neutrophils with ADP and/or ATP resulted in increased phagocytosis of IgM-EAC.

It was suggested that platelets enhance phagocytosis of IgG-EA and IgM-EAC by actions of different substances included in PRPr.

S11-5

Suppressive effects of nicotine on the defense function of human polymorphonuclear leukocytes in vitro

SUMIKO SASAGAWA, [illegible], [illegible], [illegible] Research Foundation, [illegible], Japan.

[illegible] leukocytes (PMN) are responsible for defense [illegible]. In this study, effects [illegible] PMN defense functions were investigated [illegible] tobacco smoking [illegible]. PMN were obtained from heparinized peripheral [illegible]. Release of lysosomal enzymes [illegible] PMN and production of superoxide anion were [illegible] significantly inhibited lysosomal [illegible] PMN and superoxide anion production. However, [illegible] of PMN assayed by modified Boyden chamber method [illegible] was not affected [illegible], and nicotine itself was not chemotactic for PMN. [illegible] concentrations of [illegible] was not cytotoxic. [illegible] that nicotine inhibits the microbicidal function [illegible] PMN [illegible] not effective on PMN migration to inflammatory [illegible].

S11-6

[illegible] ACTIVITY OF CONTRACTILE PROTEIN FROM NEUTROPHILS.
[illegible], [illegible], [illegible], [illegible]. The Center for Adult Diseases, Osaka, Japan.

[illegible] contractile activity of contractile protein were investigated [illegible] Ca^{2+}-sensitive myosin light chain kinase(MLCK) and phosphatase, which were purified from porcine neutrophils. And, effects of tropomyosins(TM) from arterial smooth muscles and platelets on the contractile activity were tested. In the results, it was evident that contractile activity of myosin-B from neutrophils was regulated by Ca^{2+}, which was associated with the level of phosphorylation of myosin light chain(ML) by MLCK. TMs, regardless of their orgin, enhanced [illegible] myosin ATPase activity. These results indicate that contractile activity of neutrophils is essentically regulated by phosphorylation of ML and is amplified by TM, regardless of Ca^{2+}.

S12-1

[redacted]

S12-2

TUMORICIDAL CAPACITY OF ARTIFICIALLY ACTIVATED MURINE MACROPHAGES. W.B. ATKINSON, P.A Bass, G.B. Atkinson, C.L. Black, A.M. Harvey. Winston-Salem State University, Winston-Salem, North Carolina 27110

Polymers of glutaraldehyde spontaneously generated in aqueous solution at pH 7.8 were bound to the surface of syngeneic erythrocytes collected from C57BL/6J and from SJL/J mice. Detailed studies of the interaction of macrophages collected without inducement from the peritoneal cavity, alveolar lavage fluids, or macrophages released from lung fragments by trypsin digestion with the appropriate glutaraldehyde-treated syngeneic erythrocytes (G-red cells) were made. Rosette formation with, and phagocytosis of G-red cells by macrophages from each anatomical site were confirmed by both scanning and transmission electron microscopy. The extensive degree of phagocytosis by macrophages seen by transmission electron microscopy and the cytoplasmic bridging between such macrophages and lymphocytes that contaminated our macrophage samples (by 3. to 7%) suggested that the interacting macrophages might be activated. As an index of activation, we tested the ability of macrophages previously incubated with G-red cells to kill non-altered allogeneic tumor cells. We found that such macrophages were able to kill mouse sarcoma 180 and SV-40 virus transformed kidney cells (TCMK-1). Killing of the tumor cells did not require macrophage - tumor cell contact. Growth of steroid secreting adrenal tumor cells (Y-1) appeared to be enhanced by a substance(s) secreted by macrophages that had previously ingested G-red cells. The experimental controls were 1) macrophages previously incubated without red cells, 2) macrophages previously incubated with freshly collected syngeneic red cells, 3) G-cells only, and 4) tumor cells only.

S12-3

[illegible] MACROPHAGES AND ANTI-TUMOR ACTIVITIES [illegible] AGAINST SYNGENEIC TUMOR CELLS I. [illegible] PROPERTIES OF SPG-TREATED MACROPHAGES

T. Yamada, F. Ogawa*, S. Ishizaka, Tadayuki Ito[illegible]
[illegible] Dept. of Internal Med. [illegible], Yamaguchi University, *Dept. of Immunology, University of [illegible]

We tested anti-tumor activities of macrophages treated with a novel [illegible] Sizofiran (SPG), against syngeneic and allogeneic tumor cells. SPG was [illegible] no stimulant which was not mitogenic to lymphocytes. Treatment of [illegible] to peritoneal exudate cells (PEC) with Thy1.2 monoclonal antibody and guinea [illegible] did not affect the capabilities of tumor-cell growth suppression by [illegible] and effector-to-target contact seemed to be necessary for effective tumor [illegible] inhibition. Murine peritoneal adherent cells harvested 4 days after a [illegible] tion of SPG showed the most prominent cytostatic and cytotoxic anti[illegible] tivities. Larger SPG-treated macrophages showed most remarkable anti-tumor [illegible] and nonadherent peritoneal cells incubated with SPG did not secrete [illegible] that rendered macrophages cytotoxic. SPG at a high concentration made [illegible] adherent cells and bone-marrow-derived macrophages cytotoxic to a moderate [illegible] could induce production of IL-1-like factor to a moderate degree. [illegible] molecular structure is well elucidated, will provide a clue to [illegible] the mechanism of macrophage activation both in vitro and in vivo and [illegible] potential for clinical application to cancer therapy.

S12-4

[illegible] OF TUMOR METASTASIS WITH ACTIVATION OF MACROPHAGES [illegible]

[illegible], M. Yui, K. Yamada, T. [illegible], [illegible] at the [illegible], [illegible] Kusama, Tokushima, [illegible]

[illegible] effect of a bacterial cellular component, S-[illegible] [illegible] (S-CWS) and plant polysaccharides such as lentinan and [illegible] [illegible] biological response modifiers (BRMs), on pulmonary micrometastasis of [illegible] Lewis lung carcinoma (LLC) was examined. As a model of pulmonary micrometastasis, [illegible] cells were implanted into foot pads of [illegible] mice, and the implanted [illegible] removed 9 to 10 days later. The inhibitory [illegible] of [illegible] was evaluated from the number of pulmonary [illegible] nodules 3 weeks after [illegible] implantation. S-CWS, lentinan or SPG was found to have antimetastatic effect, according to their dose and time of injection. Three intravenous [illegible] of S-CWS after removal of the implanted tumor significantly inhibited pulmonary metastasis. A single injection of 5 mg/kg or 7 daily injection of same dose of lentinan and a single injection of 100 mg/kg or 7 daily injections of 20 mg/kg of SPG also markedly inhibited. Combined therapy with cyclophosphamide with these BRM markedly prolonged the survival of mice with pulmonary micrometastases.

Enhancement of the in vitro cytotoxic activity of peritoneal exudate cells, [illegible] alveolar lavage cells or macrophages in the lung was noted in mice treated with S-CWS, lentinan or SPG on day 5 or 7 after a single injection, respectively. Intravenous transfer of peritoneal macrophages activated with these substances [illegible] the development of pulmonary micrometastases. Inhibitory mechanism of pulmonary micrometastases and activating mechanism of tumoricidal macrophages by BRM are discussed.

S12-5

ANTIMICROBIAL ACTIVITY OF TUFTSIN, AN IMMUNOMODULATING PEPTIDE HORMONE. K. NISHIOKA, D.J.J. CHU, G. LOPEZ-BERESTEIN, R.L. HOPFER, M.M. ROMSDAHL. *The Univ. of Texas System Cancer Center, M. D. Anderson Hospital & Tumor Institute, Houston, TX 77030. U.S.A.*

Tuftsin (Thr-Lys-Pro-Arg) is a naturally-occurring hormone-like peptide presumably released from leukophilic IgG by the action of two enzymes, a protease on leukocyte membrane and a splenic tuftsin endocarboxypeptidase (Nishioka et al. Biochem Biophys Res Commun 47:172, 1972). The absence of the latter enzyme may relate to reduced levels of tuftsin in splenectomized hosts. In addition to the stimulation of phagocytosis by neutrophils and macrophages, we have demonstrated that tuftsin binds specific receptors on monocyte-macrophages, neutrophils and NK cells, and enhances their cytotoxicities against tumor cells. Since sepsis in splenectomized patients and fungal infections in patients with congenital and acquired immune deficiencies are life threatening, we have examined the antimicrobial effect of synthetic tuftsin in relevant murine models. Three months after splenectomy, DBA/2 mice were subjected to pneumococcal sepsis (i.v. injection of 10^2 Streptococcus pneumonia type III). Tuftsin-treated mice had significantly greater survival than untreated mice. Hale-Stoner mice were treated with tuftsin before being subjected to an i.v. injection of 7 x 10^5 Candida albicans 336 (a clinical isolate) on day 0, and followed up to 20 days for survival. Untreated animals died by day 5-7, while tuftsin-treated mice displayed significantly improved survival time. The above results strongly suggest the potential of tuftsin as a natural immunoaugmenting antimicrobial agent. (Supported by CA-17666, DHHS).

S12-6

DETECTION OF AN ALPHA INTERFERON MESSENGER RNA ASSOCIATED WITH INTRACYTOPLASMIC ALPHA INTERFERON ACTIVITY IN ACTIVATED HUMAN MONOCYTES. HENRY STEVENSON, GREGORY DEKABAN, SREEPTIE BENYAJATI, PAUL MILLER, MARK PEARSON. National Cancer Institute, Frederick, MD. 21701

Human monocytes are known to be capable of producing many distinct cytokines including alpha interferon (IFN_α) and fibroblast growth factor(s) (FGF). IFN_α secretion by monocytes can be activated with poly ICLC but not muramyl dipeptide (MDP). Conversely, FGF release can be enhanced with MDP but not with poly ICLC. Using two distinct cDNA probes for IFN_α, unstimulated human monocytes were shown not to produce detectable levels of IFN_α-messenger RNA. Monocytes activated to IFN_α secretion, however, synthesize a 1.0 kb messenger RNA species which hybridizes with our IFN_α probes. In addition, these cells produce two higher molecular weight forms of IFN_α-messenger RNA; one detected at 2.5 kb, the other at 7.5 kb. Monocytes activated with MDP to secrete FGF only synthesize the 2.5 kb form of IFN_α-messenger RNA. Analysis of interferon levels in monocyte cell lysates revealed that unactivated monocytes do not contain any cytoplasmic IFN_α activity, poly ICLC-stimulated monocytes contained high levels of IFN_α activity, and MDP-stimulated monocytes contained intermediate levels of IFN_α activity. These results indicate that a major level of control for IFN_α release exists at the gene transcription level. Moreover, the 2.5 kb molecular weight form of IFN_α-messenger RNA may code for molecules with interferon activity which cannot be released from the cell cytoplasm.

S12-7

MYELOTOXICITY IN MICE ADMINISTERED DIPHENYLHYDANTOIN. M.I. LUSTER*, A.N. TUCKER, J. HONG* and G.A. BOORMAN*. National Institute of Environmental Health Sciences, Research Triangle Park, NC 27709.

Myelotoxicity occurred in female B6C3F1 mice following exposure to the anticonvulsant drug diphenylhydantoin (DPH). Both the multipotential stem cell (CFU-S) and the granulocyte-macrophage-committed stem cell (CFU-GM) were significantly depressed by 50 mg/kg of DPH given in 6 evenly spaced doses over a 2 week period. Bone marrow cells from control mice exhibited normal deoxyuridine (dU) suppression of ^{3}H-thymidine (TdR) incorporation. Mice on a folate deficient diet, as well as mice treated with DPH, did not exhibit normal dU suppression unless they were supplemented with folic acid. Folic acid also prevented the DPH-induced suppression of CFU-S. *In vitro* studies were performed using the CFU-GM assay, and these studies revealed a dose-related suppression by DPH, effective at concentrations as low as 2×10^{-7} M. Cell cycle studies using the ^{3}H-TdR suicide technique suggested that CFU-S from drug treated animals were not in S phase, compared to 29% in S phase from control animals. The drug effect on stem cells could be prevented both *in vitro* and *in vivo* by a variety of thymic factors, including thymosin, which is known to alter cell cycle kinetics in mice. DPH thus appears to have a direct effect on stem cells, mediated by an anti-folate mechanism, and resulting in alteration of cell cycle kinetics.

PIII-1

DERMATOPATHIC LYMPHADENOPATHY. S. ASANO, H. KANNO, H. WAKASA. First Department of Pathology, Fukushima Medical College, 5-75 Sarutsuma-cho, Fukushima, Japan

Dermatopathic lymphadenopathy (DPL) is a form of lymph node hyperplasia characterized by a predominant paracortical accumulation of interdigitating reticulum cells (IDCs) and Langerhans cells (LCs). In human DPL, irregular shaped LCs are sporadically observed in dermis and there are many IDCs, LCs and macrophages in marginal sinus and paracortical area of lymph node. IDCs and LCs show positive reaction to ATP-ase, ACP-ase, S-100 protein and Leu 6. IDCs are divided into two types by the shape of nucleus and cytoplasmic organella. Although IDCs and LCs are similar in morphology, they can be differentiated by the presence or absence of Birbeck granules. Experimentally it appears that LCs carry antigenic stimulus from the skin via the afferent lymphatics to the draining lymph node, but there are not observed remarkable increase of IDCs in lymph node like human DPL. It might be clarified how IDCs and LCs proliferate and what role they have to T lymphocytes in DPL.

PIII-2

INDUCTION OF TUMORICIDAL MACROPHAGES AND GRANULOCYTES BY THE INTRANASAL APPLICATON OF MTP-PE, A LIPOPHYLIC MURAMYL PEPTIDE

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Research Department, Pharmaceuticals Division, CIBA-GEIGY Limited, Basel, Switzerland

In rats and mice, a single intranasal application of MTP-PE, a lipophylic muramyl peptide, dissolved in phosphate buffered saline (PBS) induces tumoricidal leukocytes in the lungs at a dose range of 0.1-10 mg/kg. The tumoricidal activity is optimal one day after treatment but remains demonstrable for 8 days. If MTP-PE is applied intranasally to rats in a volume of 300 µl PBS and the lungs are lavaged one day later, tumoricidal macrophages and neutrophils are obtained, about 80% of the lavaged cells being neutrophils. It is most probable that, because of the relatively large volume (300 µl) applied, MTP-PE enters the lungs, elicits neutrophils and activates them and the resident macrophages to become tumoricidal. After separation of the effector cells on a Ficoll gradient a difference in their tumoricidal activity can be demonstrated: cultures of neutrophils kill tumor cells within 8 hours whereas macrophages need 3 days.
Using the BL16/B16 melanoma system in C57B1/6 mice, repeated intranasal applications of MTP-PE (0.1-10 mg/kg) result in a permanent cure of the treated mice indicating that circulating tumor cells have been killed and/or lung and lymph node metastases have been eradicated.

PIII-3

ALTERED CELLULAR MECHANISMS OF TUMOR RESISTANCE FOLLOWING EXPOSURE TO CARCINOGENIC POLYCYCLIC AROMATIC HYDROCARBONS (PAH). J.H. DEAN, E.C. WARD, M.J. MURRAY, L.D. CAFER, R.V. HOUSE. Chemical Industry Inst. of Toxicology, Res. Tri. Park, NC 27709.

Immunosuppression induced by PAH carcinogens has been implicated as an epigenetic mechanism in the outgrowth of initiated cells. We have demonstrated that subchronic exposure of B6C3F1 mice to PAH carcinogens suppresses humoral immunity, cell-mediated immunity (CMI), and resistance to tumor challenge which was persistent. This report focuses on the relationship between carcinogenic potential of PAHs and effects on natural and acquired tumor resistance. The carcinogenic PAHs, 7,12-dimethylbenzanthracene (DMBA), 3-methylcholanthrene (MCA), and dibenz[a,h]anthracene (DB[a,h]A) or the noncarcinogenic PAHs, DB[a,e]A and perylene were subchronically administered subcutaneously at 5, 50, 100 or 200 µg/g of body weight. Natural killer (NK) cell cytolysis, generation of cytotoxic T-cells (CTL) and macrophage functions were assessed 3-5 days after PAH exposure. Alloantigen-induced proliferation (MLC) of splenocytes from DMBA, MCA and DB(a,h)A-exposed mice was suppressed up to 90%. CTL and NK cytolysis of radiolabelled targets was depressed up to 88% and 82%, respectively, in mice exposed to the carcinogenic PAHs. Antibody dependent cellular cytotoxicity was significantly depressed by DMBA exposure, while macrophage functions were not impaired. The extent of NK suppression correlated with impaired pulmonary elimination of intravenously injected B16F10 melanoma cells, while impairment of MLC or CTL responses correlated with increased susceptibility to challenge with PYB6 sarcoma cells. Noncarcinogenic PAHs failed to depress significantly NK, MLC or CTL responses or susceptibility to tumor cell challenge. Thus, only carcinogenic PAHs suppress CMI functions which may be important in tumor resistance.

PIII-4

LYMPHORETICULAR CELLS, ENDOTOXIN (LPS) AND D-GALACTOSAMINE (D-GAL) INDUCED LIVER INJURY. J. FIERER and M. CHOJKIER. VAMC, San Diego, CA. 92161 and UCSD, School of Medicine, La Jolla, Ca.

D-gal is a hepatotoxin that has been used to study liver injury in experimental animals. Although there is evidence that D-gal is directly toxic to the liver, a number of experiments have suggested that endogenous LPS from the animals' clonic flora contributes to D-gal induced hepatoxicity. To test this hypothesis, we compared the toxicity of D-gal in LPS responsive (C57BL/6 and C3H/HeN) and LPS resistant (C57BL/10ScN and C3H/HeJ) strains by measuring serum A.L.T. levels 24 hours after an i.p. injection of D-gal 2nM/100 gm. A.L.T. levels in normal mice are 40 units/ml. The mean A.L.T. after D-gal was 400 u/l in B6 vs. 5400 u/l in B10 and 400 u/l in HeJ vs. 1200 u/l in HeN (6 mice/group). B6 spleen cells were transferred into irradiated B10 mice (650 rad), and 3 weeks later the chimeras were challenged with D-gal; mean A.L.T. level was 2500 + 90 u/l in B10→B10 controls. Irradiated B6 spleen cells (1000 rad) also transferred D-gal sensitivity. We conclude that D-gal susceptibility is not fully expressed in LPS resistant mice and that full expression of susceptibility depends upon the genotype of a radio-resistant spleen cell, not the genotype of the hepatocyte. These experiments provide further evidence that LPS plays a major role in the pathogenesis of D-gal hepatotoxicity.

PIII-5

CELLULAR RESPONSES TO LIPOPOLYSACCHARIDE IN THE MOUSE SPLEEN. H. HARA, E. MATSUSAKI, M. HASHIMOTO, M. MORIKI, AND T. YAMANE. 1st Department of Pathology, Kochi Medical School, Okoh-cho, Nankoku, Kochi 783-01 and Department of Pathology, Public Health Institute of Kochi Prefecture, Kochi 780, Japan.

The cellular response of the splenic white pulp after single injection of bacterial lipopolysaccharide (LPS) was studied using morphometry, histoautoradiography and incorporation of tritiated thymidine. DBA/2J mice and BALB/cA nude mice were used. They received a single dose of LPS and their spleens, lymph nodes, thymus, and sternal bone marrow were studied at sequential time intervals, ranging from 6 hours to 7 days. LPS induced marked cellular infiltration of the B cell areas of the splenic white pulp which was maximal at 48 hour after its injection. The labeling index increased distinctly in the B cell areas. The cellular proliferation decreased slowly and the white pulp was reorganized into cancer histology in course of time. 48 hours after LPS, nude mice received a single intravenous injection of 500 μCi tritiated thymidine and their spleens, lymph nodes, bone marrow and peripheral blood were investigated at 1, 3, 5, 12, 24, 48, and 72 hours after thymidine injection. Labeling indices and silver grain densities showed slow reduction in the white pulp by 48 hours after tritiated thymidine, while percentages of the labeled lymphocytes in peripheral blood smear and in the cortex and medullary cords of the lymph nodes showed a rapid decline. The migration of tritiated thymidine was demonstrated [illegible] with the host histoautographs. Bone marrow showed no significant [illegible] in the white pulp was similar [illegible]

PIII-6

EFFECTS OF ESTROGEN ON RES, WITH SPECIAL REFERENCE TO HEMOPOIESIS. T. HAYAMA, Y. NAWA, M. KOTANI. Department of Anatomy, Kumamoto University Medical School, 2-2-1 Honjo, Kumamoto 860.

Although estrogenic hormones are known as a potent RES stimulator, there is no settled view as to their effects on the hemopoietic system. In the present study, effects of a single pharmacological dose of estriol on hemopoietic systems were examined in adult male (C57BL/6 × DBA/2)F$_1$ mice. Five days after i.p. injection with 10 mg estriol, many focal areas of hepatic hemopoiesis were observed. At this time, the number of nonparenchymal cells in the liver markedly increased, while the cellularity of the bone marrow or WBC count in the peripheral blood significantly decreased. The number of focal hepatic hemopoiesis was further increased by transfusion of syngeneic bone marrow cells into estriol-treated mice. Furthermore, ^{51}Cr-labeled bone marrow cells selectively accumulated in the estriol-treated mouse liver. When the number of CFU-S was examined five days after estriol-treatment, the concentration of CFU-S in the liver markedly increased, while that in the blood or in the bone marrow decreased. In addition, estriol-treated mouse serum has potent granulocyte/macrophage colony stimulating activity (GM-CSA). The elevation of GM-CSA in the serum was maintained at least for 30 days after a single i.p. injection with estriol.

These results suggest that circulating hemopoietic stem cells are trapped in the estriol-treated mouse liver, and that estriol-activated Kupffer cells play a central role in focal hemopoiesis in the liver.

PIII-7

EFFECT OF YOSHIDA SARCOMA ON THE SANARELLI-SHWARTZMAN REACTION INDUCED BY LIQUOID. I. BUSZTIE*, G. LAZAR, S. RIBARSZKI. Institute of Pathophysiology, *Institute of Medical Biology, University Medical School, Szeged, Hungary.

According to our earlier investigations the growth of subcutaneous Yoshida sarcoma activates the granulopoetic activity of the reticuloendothelial system (RES). Since RES plays important roles in blood coagulation, especially in the clearance of the intravascular fibrin aggregates, it seemed worth while to study the effect of Yoshida tumor growth on the Sanarelli-Shwartzman reaction induced by liquoid (sodium polyanethol sulphonate). In inbred, male P Amsterdam rats weighing 180-200 g, liquoid (Hoffman-La Roche, Basel) in a dose of 4 ml/100 g body weight, iv, induced in 90% of the animals generalized Sanarelli-Shwartzman reaction with bilateral renal cortical necrosis; however, the same dose of liquoid in rats bearing subcutaneous Yoshida sarcoma caused only minimal morphological alteration in the kidneys and only in 25% of the animals. Since liquoid induces severe thrombocytopenia and fibrinogen depletion not only in the control but in the rats bearing subcutaneous Yoshida sarcoma, the refractoriness of these animals may mainly due to the stimulatory effect of tumor growth on the reticuloendothelial activity. This is supported by the fact that other reticuloendothelial stimulants, such as zymosan, triolein, or endotoxin, are also effective in preventing the generalized Sanarelli-Shwartzman reaction induced by liquoid. These studies support the role of the RES in the protection against the consequences of the intravascular coagulation.

PIII-8

AUGMENTATION EFFECT OF MURINE INTERFERON-α, β ON HYDROXYL RADICAL PRODUCTION IN MURINE MACROPHAGES. M. ITO, A. ISHIDA, S. SHIOTA, R. KARMALI*, M. KRIM*
Department of Bacteriology, Fukushima Medical College, Fukushima 960, JAPAN.
* Interferon Laboratory, Memorial Sloan-Kettering Cancer Center, New York NY U.S.A.

murine macrophages (MPs), pretreated with homologous interferon (IFN)-α, β for 6-24 hr, augmented chemiluminescence (CL) considerably, when stimulated by 4-β-pherbol, 12-β-myristate, 13-β-acetate. For 48 hr preincubation, the CL was not augmented.

In reactive oxygen species, $OH^{\cdot}$ production was increased in IFN treated MPs, however the levels of O_2^- and H_2O_2 generations did not change between IFN treated and non-treated MPs.

Our results also suggest that the $OH^{\cdot}$ production is due to the lipoxygenase pathway of arachidonic acid metabolism.

PIII-9

MORPHOLOGICAL CHANGES OF HUMAN MACROPHAGES IN PATIENTS WITH OVARIAN ADENOCARCINOMA AND ITS CHARACTERISTICS

Minoru Kaneko, Hirokazu Iwasaki, Department of Obstetrics and Gynecology, University of Tsukuba, Ibaraki-mura, Ibaraki, Japan

Morphological and characteristic evidences of the macrophages were investigated in the following processes in patients of advanced ovarian carcinoma.

a) Morphological changes using scanning electron microscope (SEM)

Morphological changes of human peripheral monocytes and peritoneal exudate macrophages derived from stage III ovarian carcinoma were studied. Macrophages in ascitic fluid were already shown the characteristic ruffles in the cell surfaces. In view of the fact, peripheral monocytes were incubated with peritoneal fluid of the same individuals. Morphological alterations under SEM indicated gradually pseudopodia, enlarged petal-like ruffles and spreading after 1 or 2 hr of incubation.

b) Assay for glucose consumption

Glucose content was measured using a "Glucose-B-test Wako" kit. Peritoneal exudate macrophages from normal guinea pigs treated in vitro with ascitic fluid were activated, manifesting increased glucose consumption.

These results indicate that provable interactions of macrophage activating factor (MAF) exist in the ascitic fluid of advanced ovarian carcinoma and will be an indicator of activation which changes the monocyte into the macrophage.

PIII-10

ALCIDE, AN ANTIMICROBIAL THAT CONTROLS WOUND FIBROPLASIA. A.J. KENYON, D.M. DOUGLAS, S.G. HAMILTON. Univ. Connecticut Health Center, Farmington, CT. 06032.

Alcide, a topical antimicrobial has been observed to reduce collagen formation in incised dermal wounds, limit wound sepsis and permit rapid epithelialization. The antimicrobial activity is dependent upon generation of chlorine dioxide from Alcide components. Experiments have been undertaken to establish the effect of chlorine dioxide on chemotaxis and on collagenase inhibition. Histologic evaluation of full thickness incised mouse (CD-1,♂) wounds and guinea pigs (Hartley) with wounds increasing in postoperative age up to 96 hrs. which had either been treated with isotonic saline, Alcide or glucan revealed that Alcide treated wounds had fewer inflammatory cells at 48 hrs. and at 96 hrs. had little evidence of collagen filling the dermal-wound gap, however, the basal cell layer and epithelium were closed. Glucan stimulated wounds had greater levels of monocytes in 48 hrs. and fibroplasia at 96 hrs. with increased wound breaking strength. A profile was obtained of proteins sequentially eluted by short-pulse ultrasonication of tissues containing wounds varing from 24 to 96 hrs. in age.

Polyacrylamide gel electrophoresis of these eluates revealed an increase in bands corresponding to collagen when wounds were treated with α_1-1 protease inhibitor and a decrease in wound strength. Alcide caused a decrease in ^{14}C-proline uptake and reduced wound strength. This data suggests both reduced chemotaxis and collagenase activity may be responsible for restricted fibroplasia. Grants from the University of Connecticut Research Foundation and Alcide Corporation supported this work.

PIII-11

PIII-12

PIII-13

[illegible]

PIII-14

RECOGNITION OF FOREIGNESS BY PHAGOCYTES AS OBSERVED BY THEIR RESPONSE TO BIOLOGICAL RESPONSE MODIFIERS. K. MORIKAWA, S. ABE, M. YAMAZAKI, D. MIZUNO. Faculty of Pharmaceutical Sciences, Teikyo University, Sagamiko, Kanagawa 199-01, Japan.

The response of phagocytes to biological response modifiers(BRM) was investigated in vivo and in vitro. Changes with time in the population of polymorphonuclear leukocytes(PMN), macrophages and lymphocytes in the peritoneal cavity of mice after injection of 14 BRM were compared with those of conventional inducers, bacteria and tumor cells. The response of phagocytes was classified into 5 types on the basis of its duration and extent. Like bacteria, many BRM induced more PMN and macrophages than conventional inducers. Comparison of the chemical structures of BRM and the other agents tested suggested that common properties of BRM inducing a high response were their (1) non existence in the host normally and (2) inability to be digested readily by host enzymes: namely they had the quality of "foreigness". The in vitro response of PMN was also investigated by examining their cytotoxicity on tumor cells in the presence of BRM by a ^{51}Cr release cytotoxicity assay. Of 20 BRM tested, only TAK(β-glucan), P.acnes, BCG and zymosan A were found to be effective. The cytotoxic activity of PMN in the presence of these effective BRM was very high, resulting in almost 100 cytolysis at an effector to target ratio of as low as 3. All five tumor cell lines tested were lysed, while spleen and thymus cells and PMN were not lysed. The cytotoxic mediator was shown to be hydrogen peroxide. When MM46 tumor cells were injected intraperitoneally with these BRM, the tumor take was reduced significantly. These results suggest that BRM may be considered as substances that potentiate host resistance by enhancing its activity to recognize "foreigness" in the body.

PIII-15

[illegible] NEWBORN [illegible] MACROPHAGES [illegible] MURAMYL [illegible], M. D., Ph. D., [illegible] Department of Medicine, Faculty of Medicine, Kyoto University, Kyoto, [illegible]

[illegible] Both cell-mediated cytotoxicity (CMC) of newborn macrophages (NM) [illegible] to inhibit growth of tumor cells across the 0.4 μ barrier of [illegible] were lower than adult PM. Cytolytic activity was not detectable even in [illegible] NM. Both newborn and adult PM can be activated by bacterial [illegible] to lose tumor cells, but the necessary dosage of [illegible] and the time required for activation were much lower and faster in NM than in adult PM. Addition of lymphokine caused the reduction of the threshold [illegible] for adult PM activation, however this was not the case in newborn PM. Activation of adult PM was inhibited by either indomethacin or prostaglandin E$_2$, but newborn PM were insensitive. Even adult PM, however, became insensitive to these agents after they were briefly activated by BCG and a lymphokine. These results indicate that newborn PM are naturally activated to some extent to manifest their cytocidal effect on tumor cell growth. The early phase of activation seems to require some membrane metabolites through cyclooxygenase pathway, and to be [illegible] regulated by some prostaglandins which might be derived from [illegible] themselves.

PIII-16

POTENTIATION OF TUMORICIDAL ACTIVITY IN HUMAN MONOCYTES BY MURAMYL DIPEPTIDE AND ITS LIPOPHILIC ANALOG, ENTRAPPED IN LIPOSOMES. S. Mutsuura, S. Sone, Mitsumasa Ogawara, Eiro Tsubura. The University of Tokushima School of Medicine, Tokushima 770, Japan

Studies were undertaken to examine whether the tumoricidal activity of human monocytes can be potentiated by their interaction with MLV liposomes containing hydrophilic muramyl dipeptide (MDP) or lipophilic muramyl tripeptide (MTP-PE). Human monocytes harvested from healthy donors and separated by discontinuous gradient centrifugation and adherence were highly cytotoxic to allogeneic melanoma cells. After 4 days incubation of these monocytes in medium, they showed little tumoricidal activity. MDP or MTP-PE was encapsulated within multilamellar (MLV) liposomes composed of phosphatidylcholine-phosphatidylserine. Freshly isolated monocytes incubated for 24 hr with liposomes containing MDP or MTP-PE remained tumoricidal during culture for up to 5 days. Moreover, the cultured monocytes were rendered tumoricidal by interaction for 24 hr with MDP or liposomal MDP or MTP-PE. About 1600 times lower concentration of MDP entrapped in liposomes than of free MDP in the medium was effective for rendering monocytes tumoricidal. Similarly, about 80 times lower concentration of MTP-PE in liposomes than of free MDP was effective for the activation of monocytes. Examination of the uptake by monocytes of liposomes containing fluorescent quinacrine showed linear correlation between the amount of liposomes added to monocyte monolayers and their phagocytosis. It is concluded that MLV liposomes containing MDP or MTP-PE are far more efficient in potentiating the tumoricidal activity of human monocytes than unencapsulated, free MDP. (Supported by a Grant-in-Aid for Cancer Research from the Ministry of Education, Science and Culture of Japan).

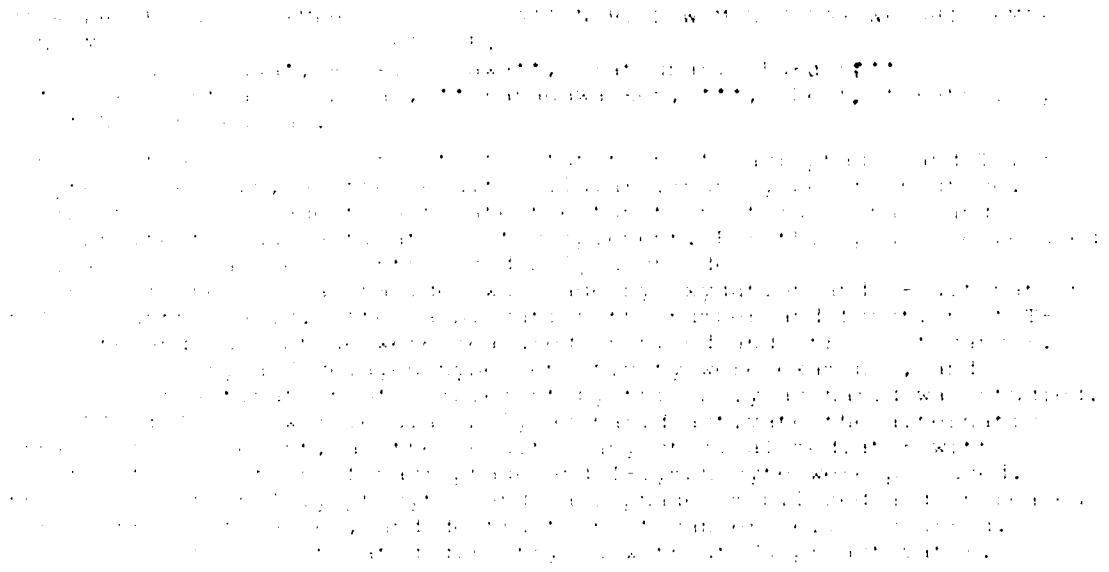
PIII-17

[illegible]

PIII-18

[illegible]

PIII-19



PIII-20

GLUCAN THERAPY ENHANCES HEMOPOIETIC REPOPULATION, INHIBITS SEPSIS AND ENHANCES SURVIVAL IN IRRADIATED MICE. M.L. PATCHEN, T.J. MACVITTIE, I. BROOK, R.J. WALKER. Armed Forces Radiobiology Research Institute, Bethesda, MD, USA 20814

Glucan is a potent reticuloendothelial and hemopoietic stimulant isolated from Saccharomyces cerevisiae. A single intravenous injection of glucan into normal mice enhances pluripotent stem cell (CFU-s), granulocyte-macrophage and pure macrophage progenitor cell (GM-CFC, M-CFC) and erythroid progenitor cell (CFU-e, BFU-e) numbers. In these studies, the ability of glucan to enhance hemopoiesis in animals hemopoietically compromised by irradiation was assayed. C3H/HeN mice were injected with 1.5 mg of particulate glucan either 1 day before, 1 hour before or 1 hour after exposure to 6.5 Gy of cobalt-60 radiation. On subsequent days, the recovery of bone marrow and spenic CFU-s, GM-CFC, M-CFC and CFU-e were assayed. In all instances, hemopoietic repopulation commenced earlier in glucan-treated than in radiation control mice; the most enhanced response occurred with glucan administered 1 day prior to irradiation. Mice were also treated with glucan before or after exposure to an otherwise lethal (9.0 Gy) dose of cobalt-60 radiation. In these experiments, only animals treated 1 day prior to irradiation exhibited increased survival (55% survival). When the livers and spleens of these radiation controls and glucan-treated mice were assayed for the presence of bacteria, radiation controls exhibited significant bacterial colonization from 11 days post-irradiation to death. By contrast, bacteria were rarely detected in organs from glucan-treated mice. Thus, it appears that glucan's ability to enhance survival following lethal irradiation may be related not only to its ability to enhance hemopoietic recovery, but also to its ability to enhance resistance to bacterial invasion which secondarily occurs following radiation insult in the hemopoietic syndrome dose range.

PIII-21

[illegible]

[illegible]

[illegible]

PIII-22

ACTIVATION OF [illegible] BY ACTIVE MANNAN FROM BAKERS' YEAST. S. [illegible], K. HASEGAWA, and M. SUZUKI.

[illegible]

Active [illegible] baker's yeast mannan containing phosphate and peptide (designated as WAM O^{6}) was found to be growth-inhibitory against WM4 and Meth-A sarcoma tumor transplanted in C3H/He and BALB/c strain mice, respectively. In order to analyze the mechanism of antitumor effect of WAM O^{6}, PMN and MΦ obtained from PEC of mice pretreated with WAM O^{6}, 1 mg/kg/day 5 times, were compared for their cytolytic effect against WM4 cells in vitro. The results clearly indicated that MΦ of WAM O^{6}-treated mice exerted stronger effect than MΦ of untreated mice (Fig. 1), and that PMN in mice treated with WAM025 did not show cytotoxic effect. PMN of WAM O^{6}-treated mice generated larger amount of superoxide than MΦ of WAM O^{6}-treated mice (Fig. 2). MΦ of WAM O^{6}-treated mice exhibited stronger lysosomal enzyme activities than those of untreated or WAM O^{6}-treated group (Fig. 3). IL-1 production was higher in PMN of WAM O^{6}-treated mice, and MΦ of the WAM O^{6}-treated mice did not produce IL-1.

These findings indicate that cytolytic effect of WAM O^{6}-treated mice is responsible for MΦ which produce lysosomal enzymes including [illegible] inducing factor, and that the activated PMN do not exert direct cytolysis to the tumor cells, although the PMN are able to stimulate T cell by releasing IL-1.

PIII-23

[illegible] LYMPHFOLLICULOID [illegible] IN [illegible]. K.TAKAKI, [illegible], [illegible], [illegible]. Department of Pathology, The Jikei [illegible] of Medicine, [illegible], Nishi-Shimbashi, Minato-Ku, Tokyo, Japan.

Kimura's disease (eosinophilic lymphfolliculoid granuloma) is a rare [illegible] mainly occurring in the soft tissue of the head and neck region and extremities. The histological features are granulation tissue accompanied by [illegible] eosinophils and mast cells and formation of lymphfolliculoid structures. The present study, applying immunohistochemistry and electron microscopy, was carried out in order to see whether the lymphfolliculoid structure was identical to the [illegible] follicle of the lymph node. The obtained results are as follows: (1) [illegible] [illegible]ally, the well-developed folliculoid structures closely simulated [illegible] follicles, but there was conspicuous irregularity of mantle [illegible] structures. (2) IgE was found to be deposited in a reticular pattern in the germinal centers. Dendritic reticulum cells (DRC) were well developed [illegible] many [illegible] multinucleated giant cells. (3) Although there were many [illegible] lymphocytes [illegible] in the germinal centers, there were much more [illegible]$^{+}$ cells and [illegible]$^{+}$ cells as compared to those found in reactive lymph nodes. It was concluded that the formation and structure of lymphfolliculoid structures in this disease was quite different from those in secondary follicles.

PIII-24

EFFECT OF [illegible] (ISO) ON THE INTERLEUKIN-1 PRODUCTION IN VITRO IN PATIENT WITH ACQUIRED IMMUNODEFICIENCY SYNDROME (AIDS). Kwong-Y. [illegible], [illegible]. Department of Basic and Clinical Immunology and Microbiology, Medical University of South Carolina, Charleston, S.C. [illegible]

Diminution of interleukin-2 production and TAC antigen (putative IL-2 receptor) positive lymphocytes (TAC^{+}) has been reported by us and others. Treatment of mononuclear cells from patients with AIDS with ISO [illegible] the production of IL-2 as well as TAC^{+} lymphocytes. The effect of ISO in vitro on the production of IL-1 were investigated in this study. IL-1 production from adherence cells were measured by indirect method using EL-4 cell line. 11 patients with AIDS were studied in this investigation. [illegible] had depressed IL-2 production. [illegible] of these [illegible] patients had depressed IL-1 production. Various concentration of ISO ([illegible] cells/ml, [illegible] cells/ml) were used to treat the adherence cells in vitro. The IL-1 production were restored to normal or near normal level in 4/5 AIDS patients. Our results indicate that depressed in IL-2 production in some AIDS patients may partly due to the depressed in IL-1 production and ISO can act as an immune potentiator in these in vitro immune assays.

PIII-25

ATYPICAL LETTERER-SIWE DISEASE WITH MARKED ERYTHROPHAGOCYTOSIS. Y. TSUNEMATSU, R. KOIKE, H. TAKAHASHI, K. SHIMIZU, S. WATANABE. National Children's Hospital, National Cancer Center Research Institute.

The relationship between the generalized form of letterer-Siwe disease, familial erythrophagocytic reticulosis and histiocytic medullary reticulosis has not been clarified. The present case was considered to belong to letterer-Siwe disease, but clinical manifestation was more similar to those of histiocytic medullary reticulosis.

The patient had seborrheic eczema on his head a few months after birth. At 1-year old, he was noticed abdominal distension due to hepatosplenomegaly and severe anemia. A scalene node biopsy revealed zonal proliferation of $S100^{+}$les-NCA^{-} T-zone histiocytes, and he was treated with PSL and VLB under a diagnosis of letterer-Siwe disease. He was referred to the National Children's Hospital at 1 y 8-mos-old with marked hepatosplenomegaly and aggressive pancytopenia. Peripheral blood revealed marked anemia and pancytopenia with erythroblasts and reticulocytes. Coombs tests was negative, and there was no hyperlipemia. Bone marrow revealed erythroid hyperplasia and histiocytosis, in which histiocytes contained Langerhans granules.

Pancytopenia became worsened, despite of the MTX treatment, and he deteriorated with increased hepatosplenomegaly and died of herpes simplex pneumonia 7 months after the admission. Autopsy revealed marked hepatosplenomegaly (liver 1,330 g, spleen 600 g) and involuted thymus (5 g). Lymphadenopathy was slight and bone marrow was fibrotic. Proliferating histiocytes were characterized with various monoclonal antibodies and revealed a phenotype of $OKM1^{+}Ia^{+}$ macrophages.

PIII-26

MACROPHAGE-MEDIATED INDIRECT EFFECT OF INTERFERONS ON THE IN VIVO TUMOR CELL GROWTH. K. Ono, M. Ida, T. Shimizu, S. Muramatsu. Department of Zoology, Faculty of Science, Kyoto University, Sakyo-ku, Kyoto 606.

An interferon (IFN)-resistant tumor cell line (R1) was established from Meth A fibrosarcoma cells of BALB/c mice. R1 cells proliferate well in vitro in the presence of high units (e.g. 10,000 IU/ml) of murine IFN (L) prepared from virus-infected L-cells (L-IFN) or recombinant human IFN-A/D (A/D-IFN, Nippon Roche Research Center). Daily i.p. administrations of L-IFN or A/D-IFN for two weeks to BALB/c mice inoculated i.p. with R1 cells resulted in the reduction of R1 cell growth in the latter period of experiment. This contrasted with the case of a IFN-sensitive Meth A cell line (S1) of which growth was suppressed by IFN more acutely. The population of peritoneal macrophages (Mø) in R1-bearing mice was larger in IFN-treated than IFN-untreated mice, and the time course of the increase of Mø number seemed to parallel that of the efficacy of IFN. The labelling index of Mø after i.v. injections of ^{3}H-thymidine was increased by the administration of IFN. Mø obtained from IFN-treated - R1-bearing mice were highly effective in suppressing the in vitro R1 cell growth in a low Mø-tumor cell ratio, in comparison with those from either IFN-untreated - R1-bearing mice or IFN-treated - non-R1-bearing mice. These results indicate that the growth of IFN-resistant tumor cells can be suppressed by Mø in IFN-treated mice, and that tumor cells and IFN synergistically stimulate the recruitment and activation of Mø.

PIII-27

ULTRASTRUCTURE OF CORDAL MACROPHAGES IN SPLEENS FROM PATIENTS WITH IDIOPATHIC THROMBOCYTOPENIC PURPURA. Y.YAMASHITA, T.ISHIHARA, T.YOKOTA, M.TAKAHASHI, E.UCHINO, N.MATSUMOTO. First Department of Pathology, Yamaguchi University School of Medicine, and the School of Allied Health Sciences, Yamaguchi University, Ube, 755, Japan.

Spleens from 28 patients with idiopathic thrombocytopenic purpura (ITP) were observed histologically, immunohistologically and electron microscopically, focusing our attention on the ultrastructure of cordal macrophages. Spleens from 17 patients contained foamy cells in the red pulp, and some of them revealed immunoreactive materials for anti-platelet antibody within their cytoplasm. Electron microscopically, cordal macrophages contained platelets in varied stages of intracellular degradation, and those containing numerous myelinlike materials were estimated to correspond to the foamy cells in the light microscopy. In the remaining 11 spleens, foamy cells were rarely observed. However, many platelets were phagocytosed by cordal macrophages.

It is suggested that in case of accelerated and/or long standing platelet phagocytosis, the amount of ingested membrane constituents is beyond the capacity of lysosomal digestion, and that the incompletely degraded myelinlike materials are most responsible for the foamy appearance of these macrophages.

Friday, September 7

S13-1

THE ENHANCED RELEASE OF INTERLEUKINS AND CHEMOTACTIC CYTOKINES FROM RAT ALVEOLAR MACROPHAGES AND T LYMPHOCYTES STIMULATED WITH DUST PARTICLES. Y.OGHISO, Y.KUBOTA, A.TSUBOI, O.MATSUOKA, *D.P.HARTMANN, *E.KAGAN. National Institute of Radiological Sciences, Chiba 260, JAPAN, and *Georgetown University School of Medicine, Washington,D.C. 20007, USA

Alveolar macrophages(AM) play an important key role in induction of pulmonary interstitial fibrosis after dust inhalation. We previously observed the release of the chemotaxin from AM of asbestos-inhaled rats. Since there is little information about immunoregulatory mediators from rat AM, the present study was done to investigate the release of cytokines from rat AM as well as splenic T lymphocytes (SC) stimulated in vitro with fibrogenic silica and asbestos dusts. Normal adherent AM population was recovered by bronchoalveolar lavage, and normal SC were obtained from non-adherent population passed through a nylon wool column. Culture supernatants from rat AM stimulated with varying doses(5 to 1000 $\mu g/ml$) of dust particles induced proliferative responses of thymocytes from C3H/HeJ mice to PHA, whereas AM cultures, by co-stimulation of dust particles and LPS, enhanced proliferation of rat fibroblasts(NRK cells) as well as mouse thymocytes. Co-culture supernatants from AM and autologous SC stimulated with dust particles alone also enhanced proliferation of mouse thymocytes under the presence of mitogen. Interestingly, these supernatants from both AM cultures and co-cultures stimulated with dust particles were accompanied with chemoattractant activity to rat resident AM, as well. These interleukins and chemotactic cytokines have a significant implications regarding the pathogenesis and immunological basis of pulmonary interstitial disorders by inhaled particles.

S13-2

EARLY CELLULAR RESPONSES TO CONCANAVALIN A IN THE MOUSE SPLEEN. K. MATSUSAKI, M. [illegible], T. MORI, T. YAMANE AND H. HARA. 1st Department of Pathology, Kochi Medical School, Okocho, Nankoku, Kochi 781-51 and Public Health Institute of Kochi Pref. Kochi 780, Japan.

This study was performed to evaluate early cellular responses to concanavalin A (con A) in the mouse spleen, using morphometry, autoradiography and incorporation of tritiated thymidine. ICR/JCL mice, 5 to 7 weeks of age received a single intravenous injection of 400 μg con A in 0.2 ml saline and were killed at various time intervals, ranging from 6 hours to 8 days. 100 μCi tritiated thymidine was administered intravenously 1 hour prior to sacrifice. Con A produced marked enlargement of the spleen. White pulp with numerous blasts in the T cell zones which reached maximum intensity by 24 hours after its injection. In the autoradiograms, markedly increased numbers of intensely labeled cells were demonstrated in the T cell zones of the white pulp. The T cell zones were expanding and the B cell zones were compressed to the periphery of the follicles. The red pulp showed early loss of hematopoietic cells and engorgement with red blood cells. Marked blastic proliferation of hematopoietic cells, however, appeared in the red pulp by 24 hours and became maximal by 48 hours. Ratio of white pulp:red pulp areas and numbers of labeled cells in the white pulp decreased to almost normal limits by 4-5 days. The incorporation of tritiated thymidine per spleen and per mg spleen increased markedly and reached a peak at 48 hours after con A. This was in agreement with the histology which showed marked proliferation of blastic cells in the red pulp by 48 hours. Thymidine uptake in the thymus and lymph nodes was less prominent and showed no significant increases.

S13-3

SUPPRESSED LYMPHOCYTE PRODUCTION BY A TRANSPLANTED GRANULOCYTOSIS INDUCING MAMMARY CARCINOMA IN MICE. M.Y. LEE, G. M. FULOP, C. ROSSE. Department of Biological Structure and Medicine, University of Washington, Seattle, WA 98195.

Mice bearing a transplantable CE mammary carcinoma have greatly augmented neutrophil production coupled with marked depletion of lymphocytes in the bone marrow (Lee and Rosse, Cancer Res. 42:1255, 1982). To test whether the marrow lymphocytopenia was due to reduced rate of lymphocyte production or to lymphocytolysis the rate of appearance of newly produced (^{3}H-TdR labeled) B cells (stained for cytoplasmic and surface expression of IgM μ chains) and non-B (IgM$^-$) lymphocytes was assessed at weekly intervals after CE tumor transplantation on radioautographs of bone marrow and spleen cells prepared 0, 24 and 48 hrs after the termination of a 24-hr continuous infusion of ^{3}H-TdR. Following tumor transplantation, marrow B lymphocytes initially increased, while Pre-B cells dropped to barely detectable levels by the end of the first week and have never appeared in the spleen. Subsequently, there was a marked decrease in both marrow and splenic B lymphocytes. The results suggest that CE mammary carcinoma cause a progressively decreased rate of small lymphocyte, B cell and non-B lymphocyte production in the bone marrow which is not compensated for by S_μ splenic lymphocytopoiesis. (Supported by DOE Contract 79EV 10270)

S13-4

S13-5

[illegible] MEDIATED REGULATION OF B LYMPHOCYTE RESPONSE TO ANTIGEN, M. F. LA VIA, V. [illegible], R. [illegible], JR., Med. Univ. of South Carolina, Charleston, South Carolina, [illegible].

In vitro exposure of mouse spleen cells to sheep erythrocyte (SRBC) immunized cultures of mouse spleen cells induced a depression in plaque forming cell (PFC) responses by activating T suppressor lymphocytes. The same reduction of PFC is seen by treatment with Ab or C3 of human peripheral blood mononuclear cell (PBMC) cultures immunized with SRBC, although the mechanism of this depression has not been elucidated. These observations suggest that circulating immune complexes can influence B lymphocyte responses via an Fc-R mediated pathway. PBMC patients with rheumatoid arthritis were obtained and Fc-R+ lymphocytes enumerated to assess a possible decrease in these lymphocytes which may suggest unavailability of Fc-R for detection by in vitro labeling. SRBC-immunized cultures were also set up to examine the PFC response. In all patients studied, as compared to normal controls, there was a significant reduction in the number of Fc-R+ lymphocytes detectable by labeling with ^{125}I-Ab_2. The PFC response to SRBC was also significantly reduced in these patients. These observations suggest that B lymphocyte responses may be reduced by activated T suppressor cells induced by the circulating immune complexes which is a characteristic feature of this disease. (Supported in part by grants from the Smokeless Tobacco Research Council).

S14-1

DIFFERENTIATION OF PROTHYMOCYTES INDUCED BY THYMIC HORMONE TP-1 OR TRYPSIN. E.H. EYLAR, Dept. of Biochemistry, Medical Univ. of Puerto Rico, San Juan, P.R. 00936, and H. FUDENBERG, Dept. of Clin. and Basic Immunol., Med. Univ. of South Carolina, Charleston, S.C. 29401.

Incubation at 37 in vitro of nude mouse spleen prothymocytes, prepared by bovine serum albumin gradient centrifugation, with thymic hormone preparation TP-1 (1-10 ng/ml) for 2 hrs induced the Thy-1$^-$ to Thy-1$^+$ conversion. Cytotoxicity assay was performed by counting the number of cells killed in the presence of anti-theta serum and rabbit complement following incubation at 37 . The TP-1 was highly purified from calf thymus by heating, ultrafiltration and Sephadex G-25 chromatography. Trypsin (1-50 µg/ml) treatment of prothymocytes also induced the conversion to the Thy-1$^+$ stage. Maximal conversion (20-30 of total cells) required 120 min incubation with TP-1 or trypsin. The effect of trypsin was inhibited by prior heating or by soybean trypsin inhibitor. It has been shown that trypsin can release glycoproteins from surface membranes, and induce cell division in confluent fibroblasts. Thus we conclude that trypsin or TP-1 act by perturbation of a membrane receptor which triggers the differentiation process.

S14-2

S14-3

ISOLATION OF FUNCTIONALLY DISTINCT RAT MACROPHAGE SUBPOPULATIONS BY PERCOLL DENSITY GRADIENTS AND CENTRIFUGAL ELUTRIATION.

ROBERT B.J. BEELEN[1] and WILLIAM S. WALKER[2]
[1]Dept. Electron Microscopy, Medical Faculty, Free University, NL 1007 MC Amsterdam
[2]Div. Immunology, St. Jude Children's Research Hospital, Memphis, TN 38101

Rat peritoneal normal steady state cells were fractionated in mast cells, eosinophilic granulocytes, lymphocytes and macrophage subpopulations. Based on their buoyant densities in a discontinuous Percoll gradient five macrophage subpopulations could be isolated and as a by-product (based on their high densities) quite pure fractions of mast cells and eosinophilic granulocytes respectively were obtained. Based on their differences in cell size in a centrifugal elutriation at least seven macrophage (Mø) subsets were isolated and as by-product a very pure lymphocyte fraction could be obtained. In both separation procedures the overall viability was 90% and the cell recovery ranged from 55-100%. Electron microscopy revealed excellent ultrastructural and cytochemical preservation of the different cell types and macrophage subsets, especially after centrifugal elutriation. Functionally, the high density Mø subsets (Percoll gradient) were only slightly enriched for ADP as compared with low density Mø subsets, however, large sized Mø subpopulations (centrifugal elutriation) were dramatically more capable to mediate ADP as compared with small sized Mø subpopulations (> 8x). This difference paralleled the capacity of large Mø to phagocytose much more SRBC (microvisual evaluation) and an increase in Fc-receptor activity (rozetting assay). Preliminary experiments do indicate this is due to an increase in number of Fc-receptors per unit cell surface area in these large macrophage subsets.

S14-4

CHARACTERIZATION OF CELL LINES DERIVED FROM ADULT T CELL LEUKEMIA AND LYMPHOMA(ATL). T.HARADA[1], M.MASAOKA[1], T.KATOH[1], F.INOUE[1], F.FUKUTA[2], and S.MORIKAWA. Departments of Pathology, Otorhinolaryngology[1], and Surgery[2], Shimane Medical Univ., Izumo 693, Japan

Six virus producing and one non-producing cell lines were established. The former were divided into IL-2 dependent(D) and independent(I) cell lines. D cells were smaller and indistinguishable from normal lymphoblasts whereas I cells were larger leukemic cells. T cell differentiation antigens as well as functional IL-2 receptors were well expressed by D cells, whereas Ia antigens were always present on the surface of both D and I cells. Virus production was variable among those cell lines and showed no correlation with the expression of differentiation antigens or IL-2 receptors. Culture with medium supplemented with human cord serum had cells keep expressing those cell markers far better than with fetal calf serum without addition of IL-2. Lymphocytes infected with ATL virus in vitro and long-term cultured with or without IL-2 were examined and revealed that much of the character of ATL cells appeared on those infected cells. Thus experiments with those transformed lymphocytes might provide with materials for the analysis of mechanisms underlying malignant transformation.

A virus non-producing cell line appeared after the culture of ATL cells. They lacked ATL provirus genome, but were unique in that about 5% of cells possessed nuclear or cytoplasmic antigen which reacted with antibody present in high percentage of ATL, nasopharyngeal cancer, infectious mononucleosis and malignant lymphoma patients. This antigen seemed to be different from known EBV related antigens and its exact nature are under extensive examination.

S14-5

[illegible] OF HUMAN MONOCYTE CELL LINES BY DNA TRANSFECTION. [illegible] NAGATA, G. DING, [illegible] M. [illegible]CAMPOS. Albert Einstein College of Medicine, Bronx, NY 10461

We have generated human monocytic cell lines from peripheral blood [illegible] by transfection with both SV40 DNA mutated in the origin of replication and DNA extracted from the U937 promonocytic cell line. Polyethylene glycol was used to fuse a $CaPO_4$ precipitate of DNA with peripheral blood mononuclear cells grown in the presence of monocyte specific growth factors. Five lines have been obtained. All lines obtained phagocytize latex beads, possess Fc and C receptors. All secrete lysozyme and collagenase and stain positively for non specific esterase. In addition all the lines express DRM1 and DRM6 antigens, and express both DR and DS antigens. These lines stimulate both allogeneic and autologous mixed lymphocyte reactions. We are currently studying whether they can substitute for primary monocytes as accessory cells in antigens presentation assays.

S14-6

ESTABLISHMENT OF HUMAN MONOCYTE CELL LINES AND SECRETION OF INTERLEUKIN 1. A.J. TREVES, V. BARAK, M. YEMIN, M. HALPERIN, M. HALIMI, Y. MILNER. Department of Radiation & Clinical Oncology, Hadassah University Hospital, P.O.B. 12000, Jerusalem 91 120, Israel.

In the present study we investigated two methods for the establishment of new human monoblastic cell lines which preserve some of their ability to secrete monokines. In the first method, we have developed hybrid cell lines between human peripheral blood monocytes and the mouse myeloma cells NS1. These hybrid cell lines, which originated from a heterologous combination, maintained some of the human chromosome complement and their mixed karyotip remained stable in culture for longer than two years. Some of the hybrid cell lines secreted constitutively an interleukin 1 (IL1) activity to the culture supernatants. Biochemical and biological analysis of the secreted product indicated its similarity to IL1 activity secreted constitutively from primary cultures of human monocytes. In a second approach, we have studied the conditions for t he establishment of cell lines from patients with acute myelo-monoblastic leukemia. We have developed a method which combines the use of macrophage-feeder layer and cloning in semi-solid media to improve the rate of success in the establishment of such cell lines. By this method we have established a new mono-myeloblastic cell line which constitutively secretes an IL1 activity to the culture supernatants. In addition, higher IL1 activity was obtained following incubation of the cells with various macrophage stimulating agents. Other established myelo-monoblastic cell lines were also found to secrete an IL1 activity, but some of them also secreted dializable as well as non-dializable factors which inhibited IL1 activity.

514-7

[illegible]

S15-1

M. [illegible] MACROPHAGE NATURE OF MALIGNANT HISTIOCYTOSIS. H.KAMESAKI, K.KITA, S.DOI, [illegible] K.TAKAMI, M.Yoshioka, H.OCHINO, *M.FUKUMOTO. The First Department of Internal Medicine, Kyoto University, Kyoto and *The First Department of Pathology, Kyoto University, Kyoto.

Histochemical and immunological characterization of tumor cells was performed in order to investigate the cellular origin of malignant histiocytosis. 7 patients who were diagnosed as clinicopathologic grounds were studied for this purpose. Cell [illegible] was obtained by the Ficoll hypaque density gradient from freshly drawn heparinized peripheral blood and bone marrow aspirates. Acid phosphatase, α naphthol acetate esterase (ANAE), peroxidase, EA, EAC rosettes, EA rosettes, and sarcoma beads preincubated with human serum (HS) were estimated. Several heteroantisera and monoclonal antibodies against human monocytes, human immunoglobulins, HLA-DR antigens, and granulocyte-associated antigens, etc. were also used in immunofluorescence. In 5 patients, the tumor cells carried the cytochemical markers typical for the monocyte-macrophage [illegible] acid phosphatase, NaF sensitive α naphthol acetate esterase, lysozyme, [illegible] receptors for Fc portion of IgG and receptors for activated third component of complement. Moreover, in 4 among them, neoplastic cells were also reactive with [illegible]. In 2 patients, the tumor cells expressed only a few cytological markers [illegible] was not determined accurately. In none of our cases, the neoplastic cells were stained by [illegible]. Thus, majority of malignant histiocytosis we investigated seemed to be derived from monocyte-macrophage system, although further study [illegible].

S15-2

RAPID DIAGNOSIS FOR MALIGNANT HISTIOCYTOSIS BY BUFFY COAT PREPARATION, BONE MARROW ASPIRATION AND LYMPH NODE IMPRINT AMONG PIANELIAGIM. Division of Hematology, Department of Medicine, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok 10700, Thailand.

Malignant histiocytosis is a rapidly progressive disease. Clinical presentation usually mimics infectious diseases. Prompt diagnosis may alter the clinical course with the hope to prolong survival and eventually to cure in some. We are presenting the values of buffy coat preparation, bone marrow aspiration and lymph node imprint for rapid diagnosis in 50 patients. The diagnosis was mainly based on the presence of malignant histiocytes and phagocytic cells. These malignant histiocytes showed plemorphic appearance which may be a) lymphocyte-like with small distinct granules b) monocytoid or monohistiocytic histiocyte c) blast-like with small distinct granules d) blast-like without granules e) blast-like with vacuoles f) blast-like with granules and vacuoles. The size of these histiocytes varied from 12 μ to 30-70 μ. Phagocytic cells showed variation in maturity. The degree of cytophagocytosis were also variable.

S15-3

S15-4

BASACEPPLATION OF HISTIOCYTIC CELLS IN MALIGNANT FIBROUS HISTIOCYTOMAS
F. Bocci, J. Kleyne and J.A.M. van Unnik, Institute of Pathology, Pasteurstraat 2, 3511 HX Utrecht.

Malignant fibrous histiocytomas (MFH) manifest a fibroblastic and also a histiocytic morphology. The origin of the histiocytic tumorcells is not yet clear. From culture and electron microscopical studies it has been proposed that they could originate from undifferentiated or fibroblastic cells. On the other hand the histiocytic cells share many characteristics with monocytes, so that also a monocytic origin has been suggested. The purpose of this study was to characterize soft tissue tumors (STT) and malignant histiocytosis (MH) with several monoclonal antibodies and antisera on frozen and deparaffinized sections. None of the STT cells express monocyte specific determinants, whereas MH tumor cells were prominently positive. All of the MFH tumors express fibroblast specific determinants. HLA-Dr/Ia antigens were present on all MH, on 50% of the MFH, but not on other STT. Peanut and soya bean agglutinin bindingssites are present on MH and on a small part of the MFH cases. MH and MFH express both alpha$_1$-antitrypsin (AT) and alpha$_1$-antichymotrypsin (ACT) antigens.

Therefore we propose that the MFH tumor cells do not originate from monocytic cells but from (undifferentiated) fibroblastic cells. During transformation into histiocyte-like cells these cells can express several characteristics, which they share with tissue macrophages, i.e. which HLA-Dr/Ia, AT or ACT antigens.

S15-5

[illegible] ANALYSIS OF HODGKIN'S DISEASE. N. Mori, [illegible] Oka, H. [illegible], F. [illegible], M. [illegible]jima, University of [illegible], [illegible], Sakura, Ibaraki [illegible], Japan. [illegible] cases of Hodgkin's disease were investigated for the [illegible] lysozyme and [illegible]-antitrypsin activity with immuno[illegible]. [illegible] peripheral blood of acute monocytic leukemia and lymph nodes [illegible] investigated. The immuno-electron [illegible] study using anti-[illegible] antiserum in Hodgkin's disease revealed that most of the atypical mononuclear cells, Hodgkin cells and Reed-Sternberg cells were positive in several small [illegible] of their cytoplasm. Epithelioid cells and macrophages were also positive [illegible] localized in the perinuclear space, rER and small vesicles in [illegible] cytoplasm. With anti-[illegible]-antitrypsin antiserum, most of the atypical mononuclear cells, Hodgkin cells and Reed-Sternberg cells showed positive reaction in [illegible]. Epithelioid cell and macrophages were also [illegible] in the small vesicles and rarely in rER with anti-[illegible]-antitrypsin antiserum. [illegible] investigation of peripheral blood of acute monocytic leukemia and [illegible] pathies demonstrated that epithelioid cells, macrophages, Langhans giant cells, [illegible] macrophages and monocytic leukemia cells were positive with anti-lysozyme and anti-[illegible]-antitrypsin antisera, but interdigitating cells were negative. Because lysozyme and [illegible]-antitrypsin are considered to be markers of [illegible] phage series and because this was proven in our present study, it is [illegible] that Hodgkin cells and Reed-Sternberg cells are [illegible] originating from [illegible] phage series.

S15-6

[illegible]

S15-7

[illegible]

S15-8

[illegible]

S15-9

VIRAL INFECTION BY HTLV AND EBV IN HUMAN LYMPHOMAS. K. MARUYAMA, S. MOCHIZUKI, K. YAMAMURA, M. MIYAUCHI, I. FUKUSHIMA, N. KOSHIKAWA, T. TAKAGI, M. NAKANO*, J.D. CAMERON**, and F.C. JENSEN**. Chiba Cancer Center, Chiba 280, Japan, *Ryukyu University School of Medicine, Okinawa 902, Japan, and **Cytotech, CA 92121, USA.

The possible dual involvement of HTLV and EBV was examined in 41 non-Hodgkin's (NH) and 10 Hodgkin's (H) lymphomas. Sera of these 51 patients were tested in ELISA at 1:100 dilution to purified HTLV. Sera of 5 NH and 1 H patients were positive. Cell cultures derived from tumorous tissues obtained from 18 (14 NH, 4 H) of these patients were examined by electron microscopy. Particles resembling retrovirus were seen in 12 (10 NH, 2 H) cultures. Herpestype particles were seen in 9 (7 NH, 2 H) of these cultures. Cultures derived from 3 (2 NH, 1 H) patients whose sera gave high ELISA values to HTLV were found to produce particles resembling both retrovirus as well as herpesvirus. Varying percentages of cells of these 3 cultures reacted by the immunofluorescence with monoclonal antibodies to different proteins of HTLV, and were EBNA-positive. Results of surface marker analyses by E-R and EAC-R, immunofluorescence with different monoclonal antibodies to T-cell or B-cell surface markers, and immunobead assays showed that 100% of cells in these cultures had B-cell markers and that some numbers of cells had both T- and B-cell markers. HTLV and EBV in one of these cultures were easily transmitted to peripheral blood lymphocytes of normal adult individuals and induced unique chromosomal abnormalities. After infection, these lymphocytes exhibited remarkably enhanced growth. These results indicate that some human lymphomas particularly those with lineage infidelity may be infected dually by HTLV and EBV that are capable of transforming normal lymphocytes. The role of these viruses in pathogenesis of human lymphoma should be further investigated.

S16-1

HUMAN MONOCYTE CHEMOTAXIS: 3 POPULATIONS DISTINGUISHED BY FUNCTIONAL AND FLOW CYTOMETRIC ANALYSIS. E.J. LEONARD, A. SKEEL, E. ALTERI. NCI, Frederick, MD 21701.

Only 20-40% of human blood monocytes migrate to chemoattractants. To analyze the basis for non-responsiveness, we used a fluoresceinated tetrapeptide attractant, fMet-Leu-Phe-Ly-FITC, for both ligand binding and chemotaxis. In 5 experiments the number of monocytes that migrated to the optimal attractant concentration (10^{-9}M) was 34 + 3% of the input number. For ligand binding, cells were equilibrated at 0°C with fMet-Leu-Phe-Ly-FITC, washed, and analyzed for fluorescence by flow cytometry of individual cells. This had the advantage over bulk binding studies of determining whether all or only a % of cells bound the ligand. Binding at 0°C was complete within 20 min and was inhibited by unlabeled peptide; saturation occurred at $3x10^{-7}$M. At saturation, 53 + 3% of the monocytes had detectable ligand binding. The apparent (uncorrected for quenching) number of fluorescein molecules bound per ligand-binding monocyte was $35x10^3$. From this individual cell analysis we can define 3 populations: [1] monocytes without receptors for the ligand - about 50% of total blood monocytes; [2] ligand-binding monocytes capable of migrating to the attractant, comprising 2/3 of the total ligand binding monocytes (34% migrators/53% ligand-binding cells); and [3] the remaining 1/3 of ligand binding cells, which did not migrate. Thus, chemotactic unresponsiveness may be due to absence of ligand binding or to events subsequent to ligand-receptor interaction. We have 2 examples of the latter (diminished responsiveness, but unaltered ligand binding): [1] immature monocytes that repopulate the circulation during leukapheresis-induced monocyte depletion (Blood 62:918) and [2] monocytes after culture in autologous serum for only 2 hrs, the diminished responsiveness of which can be prevented by 10^{-6}M serotonin (Fed Proc 43:588,1984).

S16-2

DIBUTYRYL cAMP INDUCED EXPRESSION OF C5a RECEPTORS IN U937 CELLS. D.E. Chenoweth, S.S. Soderberg, and R. von Wedel. VA Medical Center, San Diego, CA 92161

The complement-derived chemotactic factor C5a anaphylatoxin binds to specific receptors found on granulocytes. Normally, the human histiocytic cell line U937, when grown in continuous culture, does not specifically bind either ^{125}I- or fluorescene-labelled human C5a. However, after culture of these cells at an initial density of 0.5 x 10^5 cells/ml for 72 hours in the presence of 1 mM dibutyryl cAMP, U937 cells express C5a receptors that may be readily detected by either ^{125}I-ligand binding assays or flow cytometry. With ^{125}I-C5a serving as a ligand probe, the C5a receptor of dibutyryl cAMP-induced cells has an apparent Kd of 1 to 2 nM. Typically, these cells express an average of 170,000 + 40,000 receptors after induction and 70 to 80 percent of the induced cells stain with fluorescene-C5a. Dibutyryl cAMP-treated cells not only acquire C5a receptors but also become responsive to this stimulus. For example, C5a promotes both chemotactic migration (ED_{50}=0.3 to 0.5 nM) and degranulation (ED_{50} for β-glucuronidase and N-acetyl-β-glucosaminidase=1.0 to 1.5 nM) of dibutyryl cAMP-induced cells. Additionally, the C5a receptor of these cells remains functionally active in both cytoplast and plasma membrane preparations. These findings demonstrate that: 1) dibutyryl cAMP promotes expression of both oligopeptide chemotactic factor (Kay, GE, et al, Inf. Imm. 41: 1160, 1983) and C5a receptors on U937 cells, 2) the C5a receptor of these cells is functionally indistinguishable from that of normal granulocytes, and 3) these cells may be extremely useful for further biochemical characteristization of the C5a receptor.

S16-3

S16-4

516-5

EFFECT OF fMet-Leu-Phe AND AUTOLOGOUS PLASMA ON ADHESION OF HUMAN POLYMORPHONUCLEAR LEUKOCYTES

TATSUICHIRO SAKATANI, Kazuo Suzuki, Satoko Sasagawa, Tetsuo [illegible] Department of Pathology, Radiation Effects Research [illegible], Hiroshima, Japan.

[illegible] chemoattractants fMet-Leu-Phe (FMLP) and autologous [illegible] (AP) on adhesion kinetics of polymorphonuclear leukocytes (PMN) were investigated in order to elucidate regulation of PMN function in inflammatory sites. Treatment by constant concentration level of FMLP had little effect on PMN adhesion velocity. On the other hand, treatment by FMLP concentration gradient markedly suppressed adhesion velocity and the suppressive effects were potentiated in the presence of AP. Treatment by 10% or 25% AP showed suppression of adhesion velocity. Low concentration gradient of FMLP markedly enhanced the suppressive effect of AP. PMN motility was shown to be stimulated by the suppression of adhesion velocity in the presence of AP or under FMLP concentration gradient. These results suggest that plasma factors and chemoattractants act together to regulate PMN function through [illegible] adhesion.

PM -1

AUTOLOGOUS BONE MARROW TRANSPLANTATION IN A PATIENT WITH LYMPHOMA TYPE ADULT T CELL LEUKEMIA. S.ASOU, K.SAKAI, K.YAMAGUCHI, T.KAWANO, K.TAKATSUKI, The 2nd Dept. of Internal Medicine, Kumamoto University School of Medicine, S.SUMIDA, National Kumamoto Central Hospital. M.YOSHIDA, Cancer Institute, Japan.

A 41-year-old female with lymphoma type adult T cell leukemia (ATL) was treated with high dose chemoradiotherapy and autologous bone marrow transplantation. She was admitted on Jan. 9, 1984, because of lt. cervical lymphadenopathy. The hematocrit was 41.5 per cent; WBC was 9,500. No abnormal cells were found in peripheral blood and bone marrow. ^{67}Ga scan disclosed only abnormal uptake of lt. neck. The lymph node biopsy revealed non-Hodgkin's lymphoma of diffuse large cell type. The lymph node cells were SRBC rosette positive T cells. The anti-ATLA(ATL associated antigen) antibody in serum was positive. HTLV proviral DNA was demonstrated in lymph node cells, but not in peripheral blood lymphocytes and bone marrow cells. From these data, she was diagnosed lymphoma type ATL(Yamaguchi et al., Blood, 1984). From the ninth hospital day she was treated with local irradiation to lt. neck(total dose 50 Gy). On the 25th hospital day the calcium was 15.0 mg/dl. From the 27th hospital day she was treated with combination chemotherapy. Prior to transplantation, she was given large dose cyclophosphamide and 10 Gy total body irradiation. On March 7, 1984, cryopreseved autologous bone marrow was thawed and infused to the patient. After transplantation she is well. Autologous bone marrow transplantation may be one of recommendable approaches for the treatment of lymphoma type ATL.

PM -2

ANTISERA AGAINST THE INDUCER FOR THE DIFFERENTIATION OF HUMAN LEUKEMIC CELLS TO MONOCYTES-MACROPHAGES. J.W. CHIAO*, K.LEUNG*. * New York Medical College, Valhalla, NY 10595 and *Ohio State University, Columbus, OH 43210.

Human myeloid leukemic cells from cell lines or patients with acute myelogenous leukemia have been demonstrated to be induced to mature by a T cell lymphokine from lymphocyte conditioned medium. In vitro maturation induction and the lymphokine activity have been assayed in liquid culture with leukemic cells. When leukemic cell line HL-60 promyelocytes are analyzed with lymphocyte conditioned medium, a terminal differentiation to monocytes and macrophages is resulted. The mechanisms involve a cessation of cellular proliferation and expressions of characteristics of maturing monocytes-macrophages including an acquisition of complement receptors, phagocytic function and mature morphology etc. Antisera against the lymphokine maturation inducer activity have been obtained in rats using the inducer as antigen isolated from serum free culture medium conditioned with PHA and alloantigen stimulated normal human peripheral blood lymphocytes. The inducer was purified after salt percipitation, DEAE, gel filtration and SDS electrophoresis and retained the full compliment of induction activity. Incorporation of the antisera into HL-60 differentiation culture resulted in a dose related blockage of the maturation development. The cessation of cellular proliferation and the mature cell expressions were both reduced by each antiserum. Isolated inducer showed no [illegible] interferon activities and the antisera did not block the antiviral activities of these interferons. The maturation inducer as a regulator for monocyte-macrophage development is suggested.

PIV -3

KARYOTYPE EVOLUTION OF THE TRANSFORMED B-LYMPHOCYTES WITH A t(8;14) S. FUKUHARA, *Y. YAMA/OKA, H. OHNO, T. KAMEZAKI, M. KANNAGI, K. KITA, K. NASU, M. NISHIGORI, H. JOSHINO, H. YAMABE. Faculty of Medicine, Kyoto University, Kyoto 606, Japan.

To evaluate the tumorigenic significance of chromosome aberrations, we examined banded-karyotypes in 12 patients, whose tumors contained clonal cells with a t(8;14)(q24;q32). One patient had diffuse mixed-cell lymphoma following common variable immunodeficiency, and the major tumor population showed peripheral T-cell properties (E-RFC, OKT-3 & -8). In this patient, mitotic cells from the lymph node were obtained only by the stimulation of T- and B-cell mitogens: PHA-respondent cells had a normal female karyotype[46,XX], and PWM-respondent cells showed the presence of two cell populations[46,XX/46,XX,t(8;14)]. The other 11 patients had various types of non-T cell malignancy, and available mitotic cells were easily obtained without any mitogens. Four patients with diffuse large cell lymphoma and one patient in the leukemic phase of follicular small cleaved-cell lymphoma had a stem line showing highly complex karyotypes. On the other hand, 6 patients with diffuse small noncleaved-cell lymphoma including Burkitt's lymphoma-leukemia had relatively simple karyotypes, and the 3 patients had some subline cells in addition to the stem line cells with a t(8;14). These findings suggest that transformed B-lymphocytes, marked primarily with a t(8;14)(q24;q32), could enhance the tumorigenic potential over host-defence mechanisms through the karyotype evolution

PIV -4

[illegible] FROM PRIMARY CULTURED MYELOID LEUKEMIA CELLS. K. [illegible], [illegible] KAWA, K. [illegible]UDEKI, M. [illegible]ANO, T. NAKAMAKI, K. IKEDA, [illegible], [illegible]. Department of Medicine, Showa University School of Medicine, Tokyo, Japan.

Macrophages induced to differentiate from leukemia cells by various compounds differed from normal macrophage in cytochemical and immunological phenotypes. We should describe biological natures of these macrophages.

Macrophages induced from acute myeloblastic leukemia (M_1 and M_2) cells by 12-o-tetradecanoyl phorbol 13 acetate (TPA) had phagocytic activities and Ic receptors. Morphological changes with TPA are remarkable in M_2 cells than M_1 cells. Macrophages induced from leukemia cells lost the ability of proliferation. In promyelocytic leukemia (M_3) and myelomonocytic leukemia (M_4), dissociation between phagocytic activity and Ic receptor was observed. Other inducers, such as retinoic acid, dexamethasone and vitamine D_3 showed different effects on each kind of acute myeloid leukemia cells. Dissociation in morphological, immunological and cytochemical phenotypes were usually seen in matured cells differentiated from leukemia cells. Morphologically intermediate form between macrophage and neutrophil were sometimes noticed.

These results might clarify the relationship and the development of various phenotypes in the maturation of macrophage and neutrophil.

PIV -5

IMPROVED RES FUNCTION, HEPATIC CELLULAR ENERGY METABOLISM AND SURVIVAL WITH ATP-MgCl FOLLOWING MASSIVE HEPATECTOMY AMONG CIRRHOTIC RATS. H. HIRASAWA, M. OGAWA, Y. OHTAKE T. KOBAYASHI, H. SATO. Department of Surgery, Chiba University School of Medicine, Chiba, Japan.

Previous studies have shown that the depressed RES function, caused by depressed hepatic cellular energy metabolism as well as decreased functioning RES mass in remnant liver, plays an important role in the development of post-hepatectomy infection. The present study was undertaken to investigate whether ATP-MgCl$_2$, to be known to improve intracellular energy metabolism, would improve RES function and survival after massive hepatectomy in cirrhotic rats. The cirrhosis in Wistar rats was produced by the subcutaneous injection of CCl_4 twice a week for 10 weeks. Two hours after 68% hepatectomy, the rats received either 12.5 μmoles of ATP-MgCl$_2$ in 1.5 ml (ATP group) or 1.5 ml of saline (saline group) intravenously. Survival was measured over a period of 7 days. RES phagocytic activity was measured using I^{125} labeled emulsion method at 24 hours after hepatectomy. In another set of animals hepatic cellular energy charge and arterial ketone body ratio (acetoacetate/β-hydroxybutyrate, AKBR) were studied at 24 hours after hepatectomy. The survival was 100% (16/16) in the ATP group and 25% (4/16) in the control group ($p < 0.005$). RES phagocytic index was 0.0096 ± 0.0007 (n=10) in the ATP group and 0.0064 ± 0.0003 (n=10) in the control group ($p < 0.01$). Hepatic cellular energy charge and AKBR were also significantly improved in the ATP group compared to those in the control group. These data suggest that the ATP-MgCl$_2$ improved RES function and survival following massive hepatectomy in cirrhotic rats probably through the improvement of the cellular energy metabolism in the remnant liver.

PIV -6

INDUCTION BY MONOCYTES OF DIFFERENTIATION OF HUMAN MYELOGENOUS LEUKEMIA CELL LINES. T. IWAMOTO, F. TAKEDA, F. KONNO. Department of Biochemistry, School of Medicine, Showa University, Hatanodai, Shinagawa-ku, Tokyo 142.

Conditioned media from lectin stimulated leukocyte populations contain a variety of factors that can regulate the proliferation and differentiation of diverse hemopoietic precursor cells. We have examined whether monocytes as well as T cells produce factors which induce the differentiation of human myelogenous leukemia cell lines. The leukemic lines, blocked at different stages of maturation were used for study. Ml-1 cells are myeloblasts; HL-60 are promyelocytes; U 937 are monocytoid cells. The cells were cultured for 3 days in RPMI 1640 medium supplemented with 10% heat inactivated FBS and test materials. Monocytes were isolated from E-rosette-depleted peripheral blood mononuclear leukocytes of normal volunteers by adherence to serum-coated dishes. Cell preparations contained 90% monocytes as determined by staining for nonspecific esterase and peroxidase, less than 1% E^+ cells. Monocyte conditioned medium was prepared from the culture of lipopolysaccharide-stimulated monocytes. Differentiation was monitored by determining the appearance and accrual of various markers normally associated with the maturation of the granulocytic and monocytic elements.

Protein factor(s) produced by monocytes induced the various differentiation-associated characteristics in human myelogenous leukemia cell lines. All lines tested were differentiated to macrophage-like cells. The characteristics of the monocyte factor(s) were different from that of differentiation inducing factor(s) from T cells or Interferon γ

PIV-7

THE RETICULOENDOTHELIAL SYSTEM OF THE SPLEEN IN IDIOPATHIC PORTAL HYPERTENSION AND SPLENOMEGALIC LIVER CIRRHOSIS. R. KAMIYAMA, K. SAITOH. Department of Pathology, Faculty of Medicine, Iwate Medical and Dental University, Iwate 028, Japan.

Eleven specimens of idiopathic portal hypertension and 11 ones of splenomegalic liver cirrhosis were examined enzyme histochemically and electron microscopically. Some specimens were studied by tissue autoradiographic methods. Thirty-six specimens obtained from patients of gastric carcinoma were used for control. In idiopathic portal hypertension, specimens in liver cirrhosis as well as control cases, the sinus reticular cell and macrophage were moderately positive activity for naphthol-AS-acetate esterase reaction with its inhibition by NaF. On the other hand, the sinus endothelial cell showed strong activity for naphthol-AS-acetate esterase reaction, and this activity was inhibited by NaF. Electron microscopically, there was no transitional form between the stromal reticular cell and the sinus endothelial cell. In tissue autoradiography, the labeling index of ^{3}H-thymidine of the sinus endothelial cell was 0.262 ± 0.066 in portal hypertension cases, 0.06 ± 0.024 in controls, respectively. However, the stromal reticular cell was only scarcely labeled in portal hypertension cases and controls. It is speculated that sinus hyperplasia in idiopathic portal hypertension and splenomegalic liver cirrhosis is produced by the proliferation of the sinus endothelial cell itself, namely, the stromal reticular cell does not seem to differentiate into the sinus endothelial cell by these findings.

PIV-8

CELL SURFACE PHENOTYPES IN HUMAN CELL LINES OF MALIGNANT LYMPHOMAS. T. KATOH[1,2], S. MORIKAWA[1], H. NAKANO[1], I. WAKIYAMA[1], I. MINOWADA[1], AND I. HARADA[3] Depts. of Pathology[1], and Otorhinolaryngology[2], Shimane Medical Univ., Izumo 693, Japan and Leukemia Research Lab.[3], Loyola Univ. Strich, School of Med., Maywood, Ill 60153

Cell surface phenotypes were investigated in seven long-term cultured cell lines derived from human malignant lymphomas (reticulum cell sarcoma, Hodgkin's or histiocytic lymphomas). We tested if cellular origin and/or stage of differentiation could be elucidated by conventional surface marker analysis and reactivity with monoclonal antibodies(moAbs). Derivation of these cell lines from non-lymphocyte lineage was confirmed by these methods. Ia-like antigen was demonstrated in five lines by two kinds of moAbs. Antigens expressed on monocyte or myelo-monocyte lineage cells were detected by OKM1 or MCS-1 on only 1/7 lines respectively but in different line. BA-1 and BA-2 which react with antigens on small fraction of bone marrow cells were revealed to react with 4/7 respectively. These results show that surface antigens detected by these moAbs are expressed independently on malignant lymphoma cell lines, and are different from those of the myelogenic leukemia cell lines.

These results might reflect the heterogeneity of the lymphoma cell lines, as we have shown by morphological, enzyme- and immuno-cytochemical studies, or alternatively this seeming heterogeneity might be ascribed to the moAbs which were produced by immunization of leukemia cells or blood monocytes and not lymphoma cells. Thus our lymphoma cell lines could be good materials for production of moAbs for the studies of lymphoma cells.

PIV-9

[illegible] OF DIAZEPAM ON 12-O-TETRADECANOYL PHORBOL 13-ACETATE (TPA)-INDUCED DIFFERENTIATION OF HL-60 CELLS. K.NAKAO, K.SASAKI, T.MITA. Aichi Medical School, [illegible], Japan.

Recently, some reports show that diazepam inhibits the differentiation of [illegible] myeloblasts to induce the differentiation of friend erythroleukemia cells. In this study we examined the effect of diazepam on TPA-induced differentiation of HL-60 cells. HL-60 cells were cultured at 5 x 10^5 ml to 1 x 10^6 ml in RPMI-1640 media containing 10% fetal calf serum. TPA (10^{-7} to 10^{-9}M) induced macrophage-like cells from HL-60 cells. These macrophage-like cells attached to petri dishes formed large aggregates, had several cytoplasmic processes and phagocytic activity. The addition of diazepam at 20-50 ng/ml and 10^{-7} M TPA to HL-60 cells kept cells round and floating with good viability (>95%). Diazepam inhibited TPA-induced aggregation of HL-60 cells. In addition, this event was accompanied by significant change in galactosyltransferase activity. These cells had [illegible] processes and insignificant phagocytic activity, similar to controls. TPA-induced differentiation was accompanied by the decrease in DNA and RNA synthesis of HL-60 cells. The addition of diazepam had tendency to inhibit the decrease in nucleic acid synthesis of HL-60 cells. These results suggest that diazepam inhibit TPA-induced differentiation of HL-60 cells.

PIV-10

[illegible] EFFECT OF A STREPTOCOCCAL PREPARATION, OK-432, ON RES FUNCTION AND [illegible] IN CIRRHOTIC RATS. T. KOBAYASHI, H. HIRASAWA, M. OCHIA, H. SATO. Department of Surgery, Chiba University School of Medicine, Chiba, Japan.

It has been shown that cirrhotic patients are susceptible to infection due to depressed RES function. The present study was undertaken to investigate whether OK-432, a penicillin-, heat-treated lyophilized powder of Su-strain Streptococcus pyrogenes A3, would improve RES function and survival following sepsis in cirrhotic rats. The cirrhosis was produced by the subcutaneous injection of CCl_4 twice a week for 10 weeks. Either OK-432, 0.1 KE/rat (OK-432 group) or saline (saline group) was injected intraperitoneally on day five after the last CCl_4 injection. Sepsis was produced by ischemic intestinal loop method two days after OK-432 or saline injection. Global RES phagocytic activity was measured using ^3H labeled lipid emulsion method prior to the sepsis procedure. In vitro Kupffer cell activity and plasma opsonic activity were also studied using liver slice bioassay method. The survival was measured over a period of 7 days. The survival was 33.3% (n=15) among saline group and 80% (n=15) among OK-432 group ($p < 0.01$). The global RES phagocytic index was 0.0492 ± 0.0067 (n=15) in saline group and 0.0544 ± 0.0041 (n=15) in OK-432 group ($p > 0.05$). The Kupffer cell activity and the plasma opsonic activity were also significantly improved among OK-432 group compared to saline group. Thus the OK-432 significantly improved the survival of cirrhotic rats following sepsis. The OK-432 also significantly improved the RES phagocytic activity through the restoration of both the Kupffer cell activity and the plasma opsonic activity among cirrhotic rats.

PIV-11

IMMUNOLOGICAL CHARACTERIZATION IN AN ADULT PATIENT WITH CHRONIC EBV INFECTION PROGRESSING TO MALIGNANT LYMPHOMA. S.SHIRAKAWA, T.KOH, I.TANAKA, K.KITA, Y.KARITANI. 2nd Dept. of Internal Medicine, Faculty of Medicine, Mie University, Tsu, Japan.

The patient, 62-year-old man, consulted our clinic due to cervical lymphadenopathy with sore throat in March 1982. His initial laboratory values showed WBC of 10,400/mm^3 with a differential of 9.4% of atypical lymphocytes, and strongly elevated EBV related antibodies (VCA-IgGx5120, EA-DR IgGx640, EBNAx40). In spite of lymph node biopsies carried out several times, histopathologic findings revealed reactive lymphadenitis with no malignancy. In September 1983 he was acutely ill, febrile with abruptly enlarged lymphadenopathy. The axillar lymph node biopsy was compatible to the diagnosis of malignant diffuse lymphoma, large cell type of B-cell origin. Also, the tumor cells were definitely EBNA positive, and EBV molecular hybridization study clearly indicated that the lymphoma cells had EBV genome. Along the clinical course the immunological states of the patient were examined several times. The following characteristics were obtained as differed from the usual cases of IM. 1) An increased cell population of OKT8$^+$ and OKIa$^-$ in the peripheral T-cell subset. 2) Normal response in NK cell activity and mitogenic response of lymphocytes. 3) Suppressor T-cells could not be induced in vitro system of PWM-induced antibody respose. 4) In the outgrowth inhibition assay used to evaluate EBV-specific cell mediated immunity, no successful inhibition was observed without addition of IL-2. Accordingly, the present study suggests that the patient might develop from chronic IM to B-cell lymphoma due to an impairment of immunological surveillance against a dire consequence of EBV infection. (A part of this work supported by a Grant-in-Aid from the Ministry of Health and Welfare in Japan.)

PIV-12

IMMUNOHISTOCHEMICAL ANALYSIS OF MALIGNANT LYMPHOMAS WITH MONOCLONAL ANTIBODIES. A. MIKATA, H. SUZUKI and H. OHKAWA Department of Pathology, School of Medicine, Keio University, Tokyo 160, Japan

Lymphomatous tissues were investigated to reveal any special relations between lymphomatous cells and lymphoreticular stromal cells. 47 lymphomas including 24 B cell origin, 16 T cell origin and 7 unidentified origin, were stained with indirect immunoperoxidase method on tissues fixed in 2% PLP or with 4 step PAP method on acetone fixed sections of fresh frozen tissues. Monoclonal antibodies employed were OKT-3, 4, 6, 8, 9, Leu 2a, 3a and 7, HLA-DR, B-1 and Ba-1.

Results indicated that follicular lymphomas showed similarities to reactive lymph follicles in that 1) center of the neoplastic follicles were HLA-DR$^+$ and Ba-1$^-$ while periphery of the neoplastic follicles were Ba-1$^+$, and that 2) intermixed T cells showed T4:T8 ratio of 2:1 or more. I6$^+$ Langerhans cells were surrounded by T4$^+$ cells and not by T8$^+$ cells. Similar relations were seen in cutaneous T cell lymphomas. I6$^+$ cells were increased in the lymphomatous skin but not in the lymph nodes. Leu 7$^+$ cell were variable in number in both T and B cell lymphomas. B cells remained in T cell tumors as a nodule. These findings may be important to clarify tumor-host relations and immunological capacities of the neoplastic lymphoid cells.

PIV-13

MARKER PROFILE AND CYTOKINE PRODUCTION BY NEW NON-LYMPHOID CELL LINE (HDLM 1-3) ESTABLISHED FROM HODGKIN'S DISEASE. J. MINOWADA, K. OTSUKA, H.G. DREXLER AND M.S. LOK. Edward J. Hines, Jr. Veterans Administration Hospital/Loyola University Stritch School of Medicine, Hines, IL 60141 and V.A. Hospital, Leavenworth, KS 66048.

Three cell lines (HDLM-1, -2 and -3) were established from pleural effusion of a patient with Hodgkin's disease. Morphologically, HDLM cells form spontaneously "Reed-Sternberg"-like multi-nucleated giant cells in 5-10% of cell population. The HDLM cell lines, however, appear to represent a single clonal tumor cell population on the basis of the presence of marker chromosomes. An extensive characterization of marker profiles of HDLM cell lines was done by a total of 41 murine monoclonal antibodies, 3 rosette assays (E,EA and EAC), 7 polyclonal antibodies (for TdT and 6 immunoglobulin L and H chains), and anti-HBV and HTLV antibodies. Based on the comparisons with those marker profiles of 84 lymphoid, myelomonocytic and erythroid leukemia-lymphoma lines available in the laboratory, the HDLM cell lines were found to be unique non-lymphoid, non-myelomonocytic, non-erythroid and non-epithelial cells. Partial expression of antigens related to suppressor T (Leu-3A), IL-2 receptor (Tac), early myeloid cell (MCS-1) and megakaryocyte-platelet (GA-2, DG-ALL-1) was observed. Neither EBV-nor HTLV-antigens were detectable. The marker profile of HDLM cells was different from that of another "Hodgkin's tumor" cell line (L-428; Diehl et al. J. Cancer Res. Clin. Oncol. 101:111,'81). Isoenzyme profile of esterase, acid phosphatase and -hexosaminidase in the HDLM cells supports uniqueness of cell type. HDLM cells produce constitutively a differentiation inducing factor of myeloblasts to monocyte-macrophage differentiation in vitro and a factor inhibitory to T-cell proliferation in vitro.

PIV-14

ENZYME CYTOCHEMICAL AND IMMUNOCYTOCHEMICAL STUDIES ON MACROPHAGE-LINEAGE CELL LINES DERIVED FROM HUMAN MALIGNANT LYMPHOMAS. S. MORIKAWA, T. HARADA, M. NAGASAKI, Y. MORIKAWA, J. KATOH AND J. MINOWADA. Departments of Pathology, Internal Medicine, and Otorhinolaryngology, Shimane Medical University, Izumo 693, Japan, and Leukemia Research Lab., Loyola University Strich, School of Medicine, Maywood, Ill. 60153

Original cells of malignant lymphomas possibly consist of lymphocyte- and macrophage-lineage cells. Immunological and biological studies of malignant lymphoma cells, especially derived from non-lymphocytic lymphomas such as histiocytic lymphomas and Hodgkin's disease, are expected to make a contribution to understanding differentiation and heterogeneity of macrophage-lineage cells.

In this study, 7 long-term cultured human malignant lymphoma cell lines are investigated enzyme- and immuno-cytochemically. As the controls, 4 human myeloid leukemia cell lines are also examined. Peroxidase, acid and alkaline phosphatase, non-specific esterases, ATPase and succinic dehydrogenase are demonstrated enzyme cytochemically. Lysozyme, α_1-antitrypsin(α_1-AT), and S-100 protein are studied immunocytochemically.

Taking together with the results of biological and surface marker studies, these malignant lymphoma cell lines are classified into 3 subgroups; S-100 protein & α_1-AT positive, Fc-receptor & ATPase activity positive, and phagocytic & lysozyme activities positive group. These observations suggest the heterogenous cellular origins of human malignant lymphomas, as well as macrophages.

PIV -15

[illegible] MONOCYTIC LEUKEMIA LINES JOSKI AND JOSKS — ESTABLISHMENT AND CHARACTERIZATION. M.OHTA, M.AKASHI, H.NOJIRI, K.MOTOYOSHI, Y.MIURA AND M.SAITO
1st.Hemopoiesis, Inst.Hematology and Dept.Hematology, Jichi Medical School, Tochigi 329-04, JAPAN.

With a very few exception, it has been difficult to establish human non-lymphoid monocytic leukemia lines. We have recently established two new monocytic leukemia lines successfully. One line, designated JOSKI, was derived from acute myelomonocytic leukemia (M4), and the other, JOSKS, from acute monocytic leukemia (M5). Leukemic cells isolated from the peripheral blood of each patient by Ficoll-Hypaque method were cultured in alpha medium with 10% fetal calf serum in 96-well microplates at 37°C in a humidified 5% CO_2 atmosphere. Each line was considered to be established by week 7 or 8 at which time it became possible to subculture the cells continuously. Both lines reached a saturation density of 1.5x10⁶/ml when seeded at 2x10⁵ ml, with a doubling time of 24-28h. In wright-Giemsa preparations, cells were round and polygonal in shape with small blebs. The cells had basophilic cytoplasm with a few vacuoles and indented nuclei with 1-3 large nucleoli. Electron microscopic studies revealed that these lines had immature monocytic features. Both lines were positive for the staining of alpha naphthyl butyrate esterase which was completely inhibited by sodium fluoride. They became adherent to plastic culture dishes and acquired phagocytic activity after induction by 12-O tetradecanoyl phorbol-13 acetate within 24h. Other phenotypic characteristics and differentiation [illegible] will be discussed in reference to the usefulness of these monocytic lines for studying the host defence mechanism.

PIV -16

IMMUNOHISTOCHEMICALLY INVESTIGATIONS OF SOFT TISSUE TUMORS, ESPECIALLY MALIGNANT FIBROUS HISTIOCYTOMAS. P.J. Schöll, J. Kleyne, J.A.M. van Unnik, J.R.J. Elbers, M.C. D. van der Vegt, Ch.F. Albus-Lutter. Institute of Pathology, Pasteurstraat 2, 3511PX Utrecht, The Netherlands.

Soft tissue tumors consist of a group of morphologically divergent tumors of mesenchymal origin. A large group of STT is formed by the malignant fibrous histiocytoma (MFH) and many of these tumorcells have a histiocytic appearance. The presence of histiocyte-specific markers within the cytoplasm of MFH tumor cells favours a dual fibroblastic-histiocytic relation. We have investigated the STT immunohistochemically for the presence of receptors for peanut- and soya bean agglutinin (PNA and SBA) and for $alpha_1$-antichymotrypsine (ACT) using the unlabelled PAP staining procedure on deparaffinized sections. Our results showed that rhabdomyosarcoma (RS), osteosarcoma (OS) and MFH could be positive. For example 58% of the subcutaneously located MFH (n=14) stained for ACT, 21% for SBA and 7% for PNA bindingsites. Deeply located MFH's (n=63) stained respectively for 36%, 25% and 11%. There was a preferential staining of giant and histiocytic cells. It was striking that a great part of the histiocytic cells in STT (RS-OS-MFH) express, but only a small part the Peanut Agglutinin - or SBA bindingsites. In contrast to malignant histiocytosis which express ACT, PNA and SBA receptors. These tumors are derived from cells belonging to the monocytic cellline. Our results showed that mesenchymal cells could behave as histiocytes with respect to morphology and the expression of ACT antigens, but that they differ from "real" histiocytes in their expression of the lectin-receptors.

PIV-17

INVASIVENESS AND METASTATIC POTENTIAL OF T-CELL HYBRIDOMAS. E.ROOS,P.DE BAETSELIER, M.J.STEWART. Netherlands Cancer Institute, 121 Plesmanlaan, 1066 CX Amsterdam, The Netherlands and Institute for Molecular Biology, Free University, Brussels, Belgium.

Alloantigen- or ConA-activated T-cells were observed to be invasive in vitro in hepatocyte cultures, similarly as highly invasive and metastatic lymphoma cells, whereas non-stimulated spleen T-cells were not. Recently, it was found that spontaneous fusion in vivo between non-invasive and non-metastatic BW thymoma cells and normal host T-lymphocytes gave rise to highly metastatic cells (manuscript submitted). We assumed that such hybrids were metastatic because they expressed the invasiveness of the normal T-cell fusion partner. To test this hypothesis, we prepared hybrids between BW cells (AKR-derived) and activated AKR T-cells, and tested their invasiveness and metastatic potential. All obtained hybridomas were highly invasive. The cell line lost invasiveness after a few weeks in culture. We also fused BW cells with normal spleen T-cells. Some resulting hybridomas were not invasive, but most did invade. To test their metastatic potential, hybridoma cells were injected into the tail vein of AKR mice. Invasive hybridomas gave rise to extensive and widespread metastasis. Livers and spleens were much enlarged and diffusely infiltrated, and large tumors were found in kidneys, ovaria and mesentery. In contrast, non-invasive hybridomas did not yield metastases. We conclude that a high level of malignancy can be conferred onto T-cell hybridomas by properties derived from normal T-cells. Because of their extraordinarily high invasive and metastatic potential, T-cell hybridomas constitute an attractive tool for metastasis research.

PIV-18

[illegible] PAPULAR VARIANT OF PAPULAR PRURITIC ERUPTION FOLLOWED FOR [illegible] YEARS.

[illegible] AFAZAFI AND [illegible] TAFA

[illegible] Dermatology, Fukuoka University Medical School, [illegible]-1-1, Fukuoka 860, Japan.

A case of pruriginous reticulosis (PR) followed for 12 years was studied histopathologically and electron microscopically. Ultrastructural studies of erythematous plaque in the early stage revealed that histiocytoid cells with abundant cytoplasm and lymphoid cells with convoluted nuclei infiltrated along the basal layer of the epidermis. In contrast to these findings of erythematous plaque, a biopsy taken from the tumor in the late stage showed different appearances. Numerous large pleomorphic lymphoid cells proliferated in the dermis but few histiocytoid cells were observed.

From above observations, we consider that histiocytoid cells were increased reactionally in the early stage and neoplastic lymphoid cells proliferated in the late stage. Therefore, PR could be a type of neoplastic lymphoproliferative disease originating in the skin such as mycosis fungoides.

PIV-19

DEVELOPMENT OF EXPERIMENTAL HEPATITIS AND FUNCTION OF THE RES. S. SASOU, F. SATODATE, T. MAIAHAME, T. MASUDA. Departments of Pathology and Internal Medicine, Iwate Medical UNIV.,School of Medicine, Morioka 020, Japan.

The function of the RES was examined in relation to the development of experimental hepatitis. Mice were divided into the following three groups; 1 Mouse Hepatitis Virus 3 (MHV) was inoculated into mice with no previous procedure in Group A, (2)in mice after blockade of the RES with carbon particle in Group B, and (3)control rats were Group C. LD50 of mice was compared between Groups A and B. The livers were also simultaneously observed morphologically with examination of the carbon clearance rate. In addition, the Kupffer cells, which have phagocytosed carbon particles, were counted in Group A. Correlation was estimated between the carbon clearance rate and the number of Kupffer cells which have phagocytosed carbon particles.

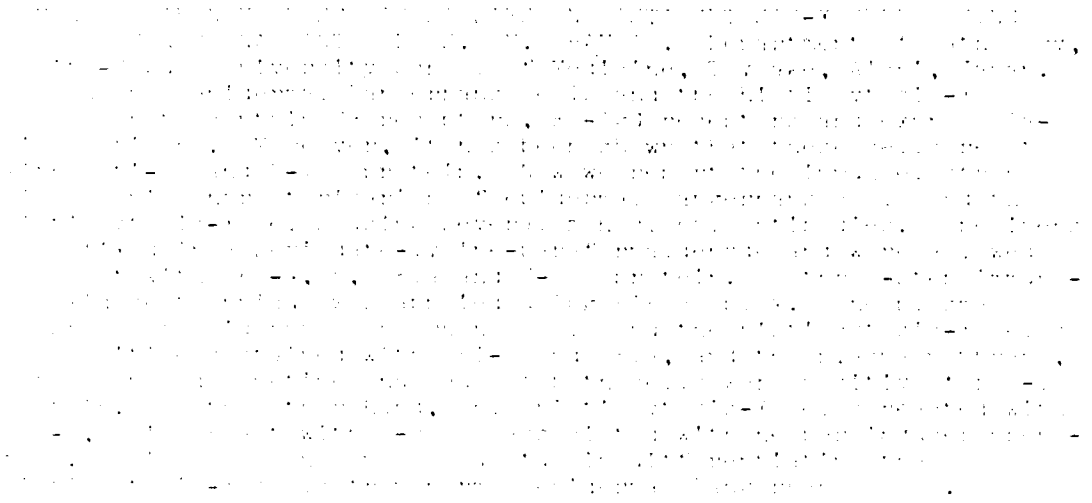
In Group A, phagocytic activities exhibited by the carbon clearance method and the number of Kupffer cells phagocytosing carbon particles were increased until 36 hours after inoculation of MHV, then decreased from 48 hours. The number of Kupffer cells was increased most markedly in the middle than in the central or peripheral zone of the lobules.

Degenerating and necrotizing liver cells appeared earlier and more severe in Group B than in Group A. It was been suggested that decrease of phagocytic activity of the RES closely relates to development or acceleration of hepatitis by virus infection.

PIV-20

[illegible]

PIV-21



PIV-22

ADULT T-CELL LEUKEMIA LYMPHOMA ON THE EAST COAST OF KII PENINSULA IN JAPAN

T.KOBAYASHI, I.TANAKA, T.KOH, K.KITA, Y.KARITANI, S.SHIRAKAWA. 2nd Dept. of Internal Medicine, Faculty of Medicine, Mie University, Tsu, Japan.

Twenty seven patients with adult T-cell leukemia lymphoma (ATLL) have been found in the last eight years along the east coast of Kii Peninsula in the middle district of Japan. Their age ranged from 27 to 88 yr with a mean of 54.6 yr and the male/female ratio was 19/8. Most of the patients had lymphadenopathy, splenomegaly, hepatomegaly and skin lesion. Hematologically, leucocytosis of more than 50,000/cmm was observed in most of the patients, but anemia and thrombocytopenia were mild in comparison with other leukemias. Immunoglobulin levels were within normal limits in most cases. Hypoproteinemia and hypercalcemia were characteristically noted in many patients. The prognosis was very poor (median survival: 79 days) and most of the patients died of pulmonary infections. The leukemic cells in the blood were characterized by marked deformation of the nucleus and the leukemic cells reacted positively with the OKT3 and OKT4 monoclonal antibodies, showing immunologically inducer/ helper T-cell phenotype. Sera from 18 patients were examined for antibodies against ATL-associated antigen (anti-ATLA) but in two patients neither anti-ATLA in sera nor proviral DNA in leukemic cells were detected. However, these two patients could not be distinguished from other ATLL pstients clinically. The characteristics of these anti-ATLA negative cases will be discussed in comparison with the other ATLL cases. (A part of this work was supported by a Grant-in-Aid from the Ministry of Education in Japan.)

PIV -23

AN AUTOPSY CASE OF IgA MULTIPLE MYELOMA ASSOCIATED WITH IMMUNOGLOBULIN STORAGE HISTIOCYTOSIS AND AMYLOIDOSIS. K.TAKATSUKI, T.KAGIMOTO, F. KAWANO, M.CHITOSE, S.OHSHIMA, K.TAKAHASHI AND M.NAITO. The 2nd Dept. of Internal Medicine and The 2nd Dept. of Pathology, Kumamoto University Medical School, Kumamoto 860, Japan.

A case of histiocytosis with L-chain accumulation (Terashima et al.: J.Jpn.Soc. RES 17: 209, 1977) and a case of myeloma accompanied by histiocytosis with IgG myeloma protein accumulation (Itagaki et al.ibid. 21: 127, 1981) were reported in Japan. The following case represents a rare association of amyloidosis and crystal-loaded histiocytosis in multiple myeloma.

A 60-year-old man was admitted because of left femoral pain. Skeletal X-ray survey disclosed multiple bone lesions and pathologic fracture of the left femur. Plasmacytoma, 4x8cm, originated from a rib was found on the left side of the chest. Serum IgA was 1,439 mg/dl and Bence Jones protein was detected in urine. Bone marrow examination showed 32 per cent plasma cells and many histiocytes containing needle-like crystals. The patient died 20 months after the first admission. Autopsy revealed amyloidosis in the left elbow, thyroid and adrenals.

Histiocytes in the bone marrow were examined by PAP and immunoelectron microscopy. Crystals in the histiocytes were considered to be IgA-K myeloma protein.

PIV -24

On the activity of phagocytosis of lymphocytic cells.

Takaaki Ueda, Nobuniko Shibata, Junsuke Yoshitake and Nobuyuki Senda. Department of Cell Physiology. The Center for Adult Diseases, Osaka, Japan.

It is reported that B-lymphocytic cells ingest latex particles or red cells. This paper shows the results of our examinations on the activity of phagocytosis of various lymphocytic cells to clarify or classify one of their characteristics.

Venous blood were gathered from 10 healthy adults and 6 B-cell leukemia patients, i.e., 4 with acute lymphoblastic leukemia (ALL) and 2 with chronic lymphocytic leukemia (CLL). Mononuclear cells were obtained by gradient sedimentation, and suspended in Medium 199 at a concentration of about 1×10^6/ml. 5×10^7/ml of polyacrylamide beads coated with rabbit anti-human immunoglobulins antibodies (2-5 μm in diameter, Immunobeads; IB) in Medium 199 solution were mixed with cell suspension, and left standing at 20° for 15 min. After incubation at 37° for 60 min., the mixture were used for microscopic examinations. Two hundred cells were counted twice in each sample to determine the percentage of cells which ingested IB under a phase contrast microscopy.

Fourteen to 25 % (average 19.9 %) of normal lymphocytes ingested IB. All six cases with ALL did not ingest IB, but one out of two cases with CLL, 24 % of cells ingested IB.

PIV-25

[illegible] MICROSCOPIC AND KARYOMETRIC STUDY ON NON-HODGKIN'S LYMPHOMA [illegible] REFERENCE TO NUCLEAR IRREGULARITY.

[illegible]SHIMA, K. SAITO, T. UNO, T. MASUDA* AND T. WATANUKI. Dept. of [illegible]ology, Akita Univ. School of Medicine, Akita and Dept. of Pathology, [illegible] Univ. School of Medicine, Sendai(*).

The present study was undertaken to elucidate morphological differences between T cell lymphoma(TCL) and B cell lymphoma(BCL). 48 of 107 non-Hodgkin's lymphomas were examined electronmicroscopically, and 29 (7 cases of TCL and 22 cases of BCL) further submitted to computerized karyometry(Videoplan). Nuclear irregularity was estimated in terms of shape constant $K=4\pi(S/L^2)$, where S, L are nuclear area and [illegible] of nuclear section is a circle, the value of K equals 1, and increases with increasing nuclear irregularity. The results are as follows: 1) In small lymphocytic lymphomas, the value of mean K was 0.620(s.d.: 0.065) for BCL, while it was 0.620(s.d.: 0.190) for TCL, a significantly smaller value for TCL. b)In large cell lymphomas, K was 0.[illegible] for [illegible]plasma. Then, the value of K reduced for [illegible]clear, DLnc, [illegible] and [illegible]polymorphous in this succession. In general, the nuclei of TCL were more irregular than those of BCL. 2)Nuclear pockets were observed more often in lymphomas with high nuclear irregularity. 3) Golgi zones were abundant in BCLC and rER developed well in [illegible]BLplasma. Labyrinthinous structures of cell membranes were found only in BCL of follicular center cell origin.

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